

Evolution: Von der Chemie zur Molekulargenetik

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and

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Fallstudien zur naturwissenschaftlichen Erkenntnis

Wien, 17.11.2015

Web-Page für weitere Informationen:

<http://www.tbi.univie.ac.at/~pks>

Chemische Evolution

Biologische Evolution

Von kleinen Molekülen zu molekularen Replikatoren

1. Woher kommen die Bausteine des Lebens?
2. Der Ursprung der Chiralität
3. Einfache Metabolismen und Recycling
4. Ribonukleinsäuren - RNA-Welt
5. Desoxyribonukleinsäuren und Proteine

Woher kommen die Bausteine des Lebens?

H₂, H₂O, NH₃, N₂, H₂S, CH₄, CO, CO₂, Metallionen, ...



Chemie der präbiotischen Erde

Bausteine der Biopolymeren: Aminosäuren,
Nucleobasen, Kohlenhydrate, ...



Polykondensationsreaktionen

Polymere mit ungeordneten Bausteinfolgen, ...



Polymerisation an Vorlagen: Instruierte Polymere



Autokatalyse: Reproduktion von Molekülen



RNA Welt: Beginn der Darwinschen Evolution



Präbiotische Chemie:
Von kleinen Molekülen zu
molekularen Replikatoren

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← Chiralität

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Primitiver Metabolismus

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Bausteine der Biopolymeren: Aminosäuren, Nucleobasen, Kohlenhydrate, ...

Polykondensationsreaktionen

Polymere mit ungeordneten Bausteinfoolgen, ...

Kompartimentalisierung

Polymerisation an Vorlagen: Instruierte Polymere

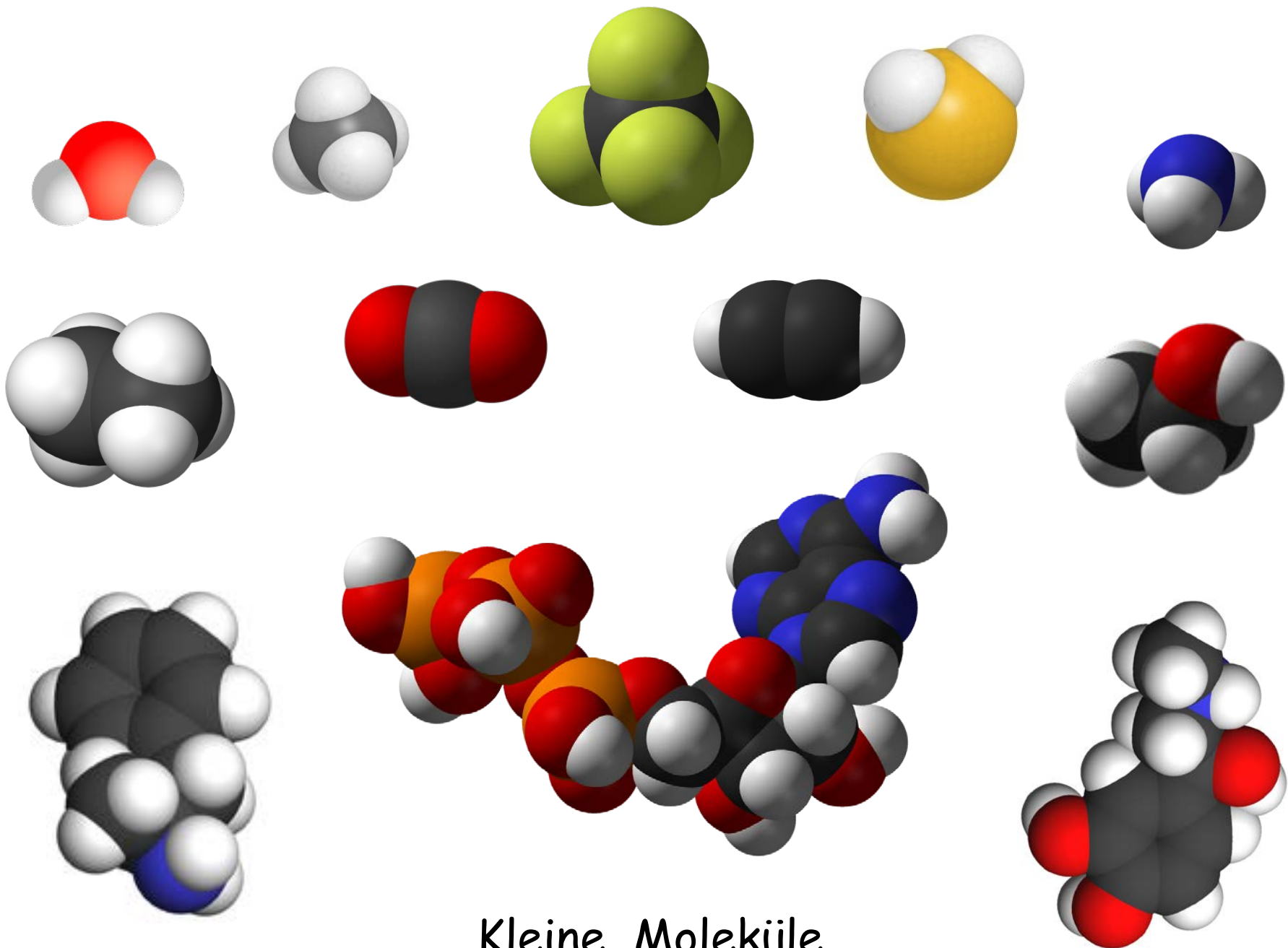
Chiralität

Instruierter Metabolismus

Autokatalyse: Reproduktion von Molekülen

Präbiotische Chemie:
Von kleinen Molekülen zu
molekularen Replikatoren

RNA Welt: Beginn der Darwinschen Evolution



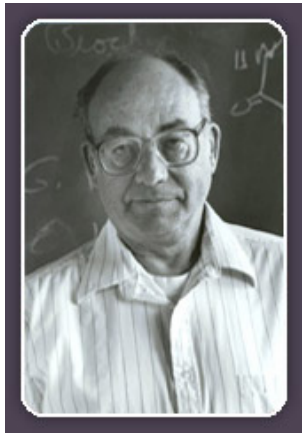
Kleine Moleküle



„Horsehead“ nebula in orion contains a huge dark cloud

Elektrische Entladung in einer reduzierenden Atmosphäre:

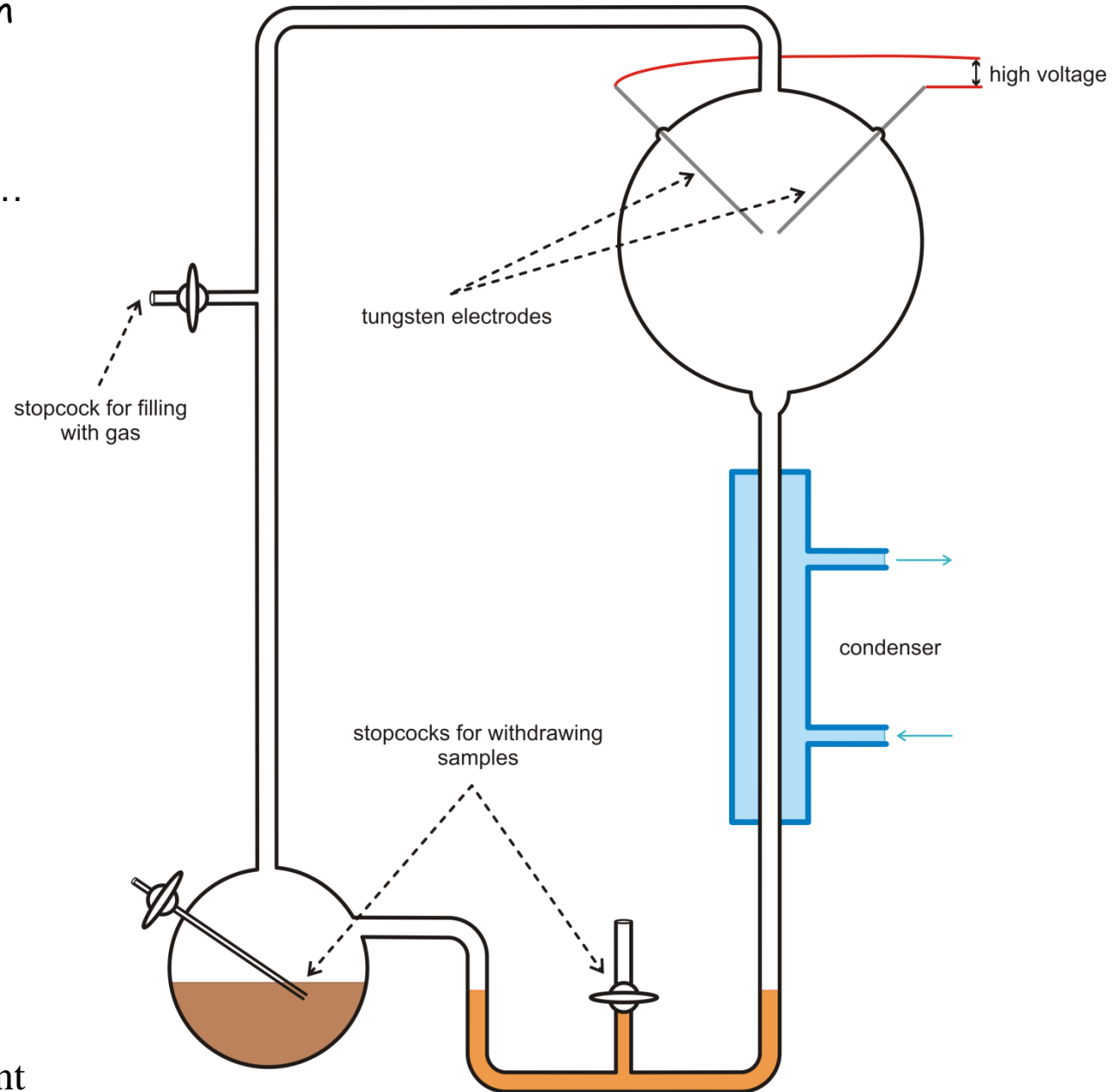
CH_4 , CO , NH_3 , H_2O , H_2 , ...

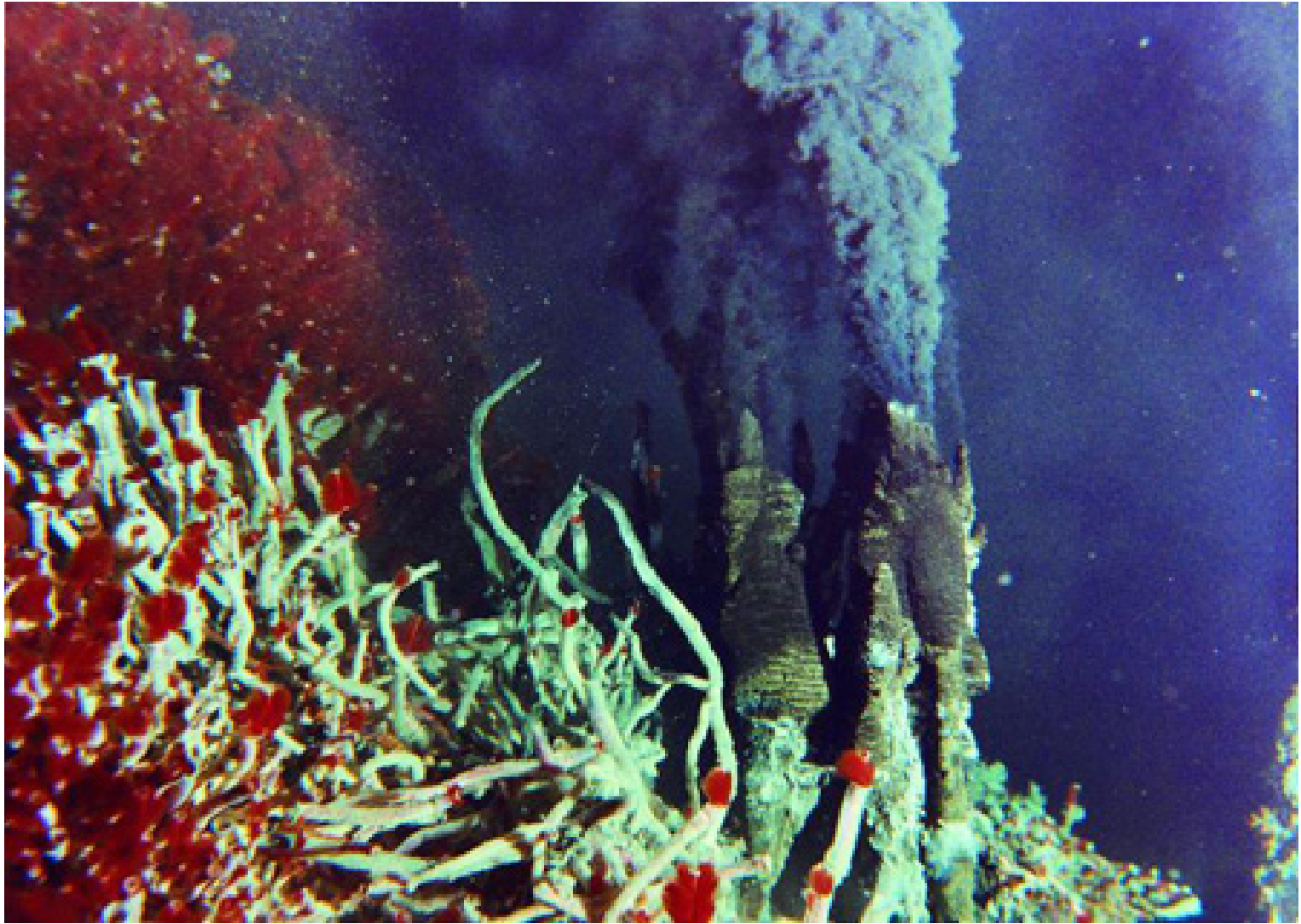


Stanley Miller, 1930 - 2007

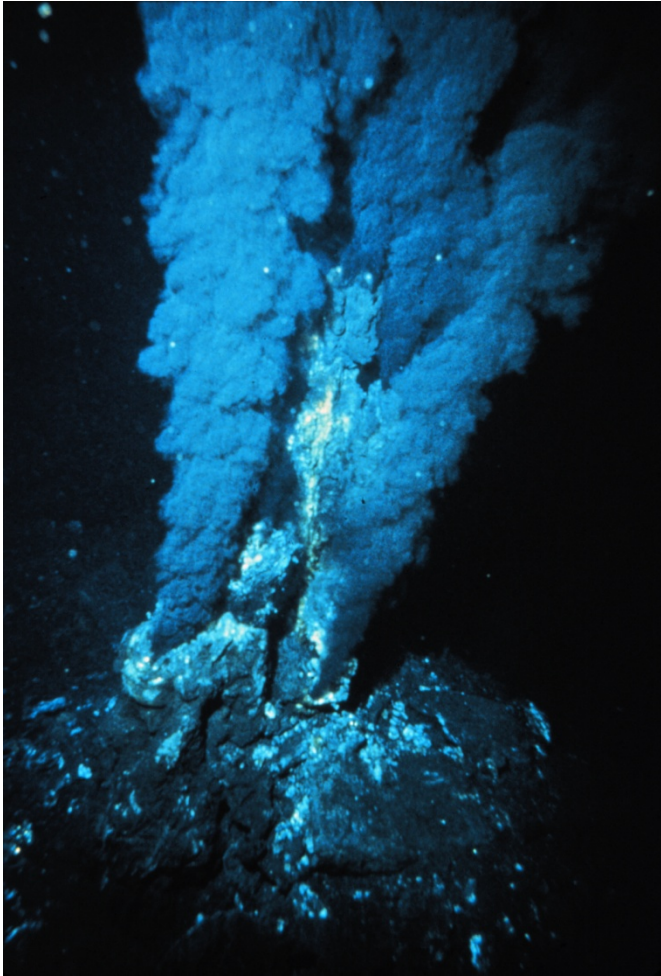
S.L. Miller. 1953. A production
of amino acids under possible
primitive earth conditions.
Science **117**:528-529

Das Miller-Urey Experiment

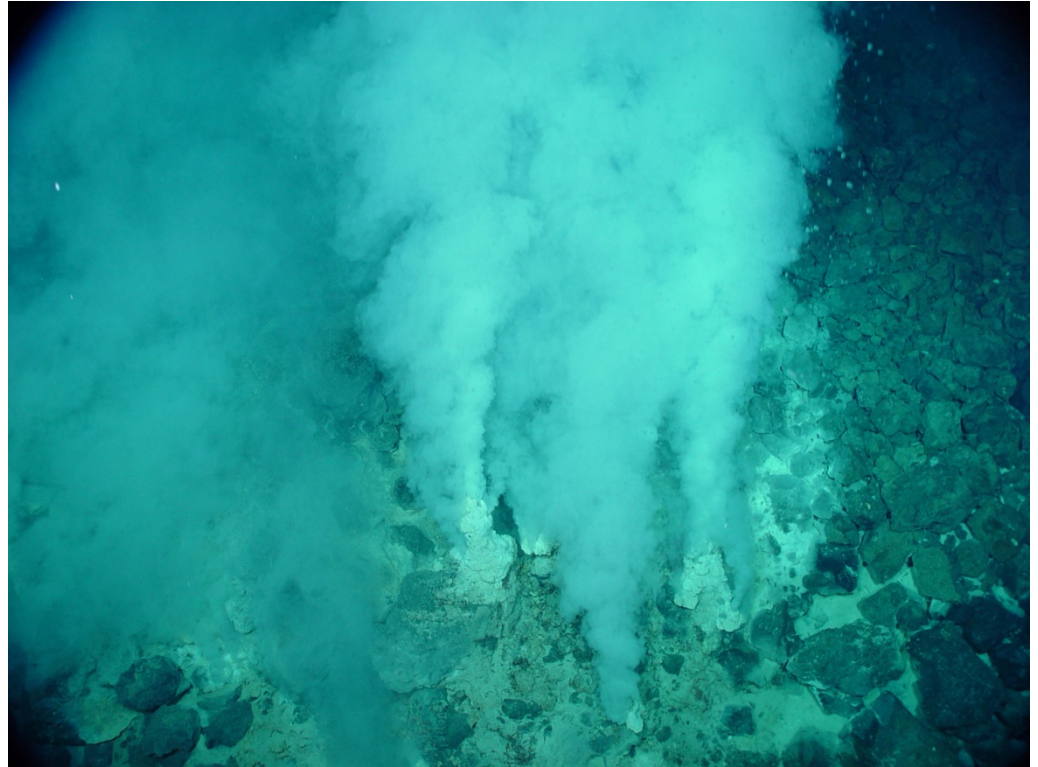




A hydrothermal vent



black smoker

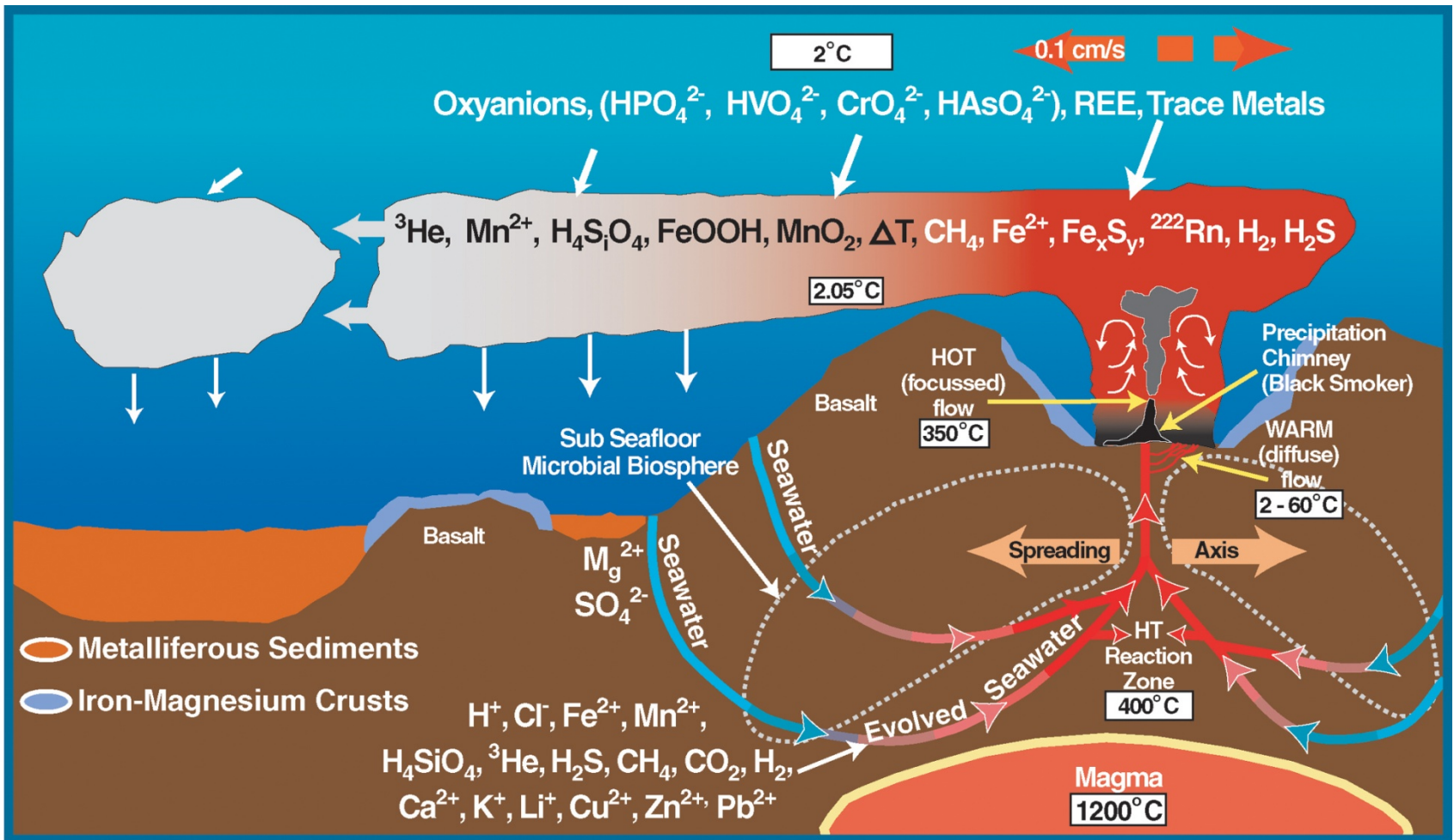


white smoker

Hydrothermale Quellen in der Tiefsee

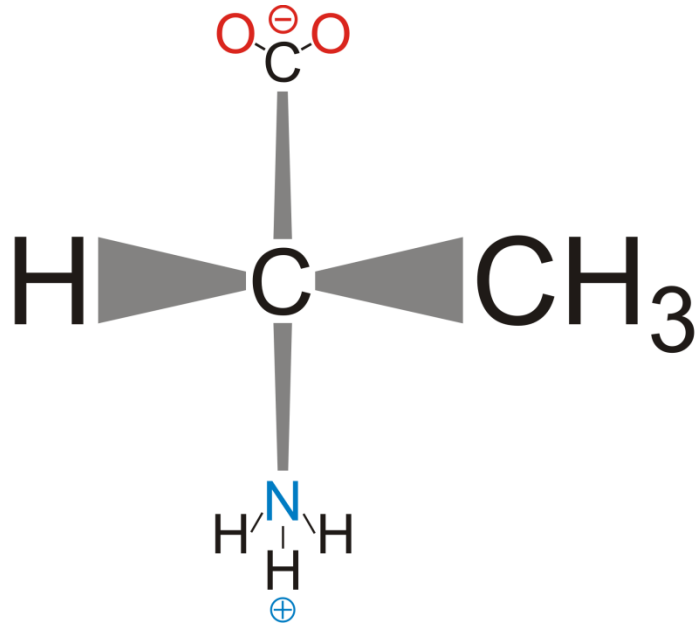
Vorkommen: mid-atlantic ridge, east pacific rise, ...
in etwa 3000 m Tiefe

Source: Wikipedia: *Hydrothermal vent*, Nov. 15,2011

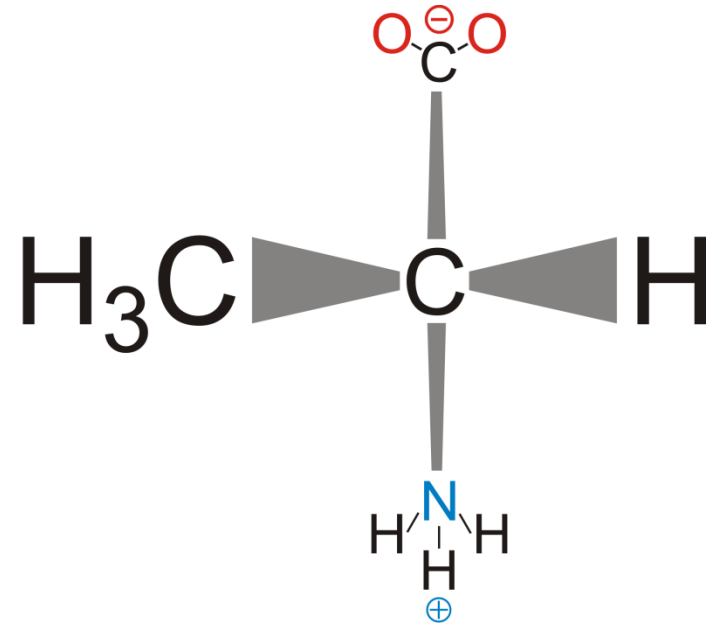


Bedingungen und Materialien in und um hydrothermale Quellen

Der Ursprung der Chiralität

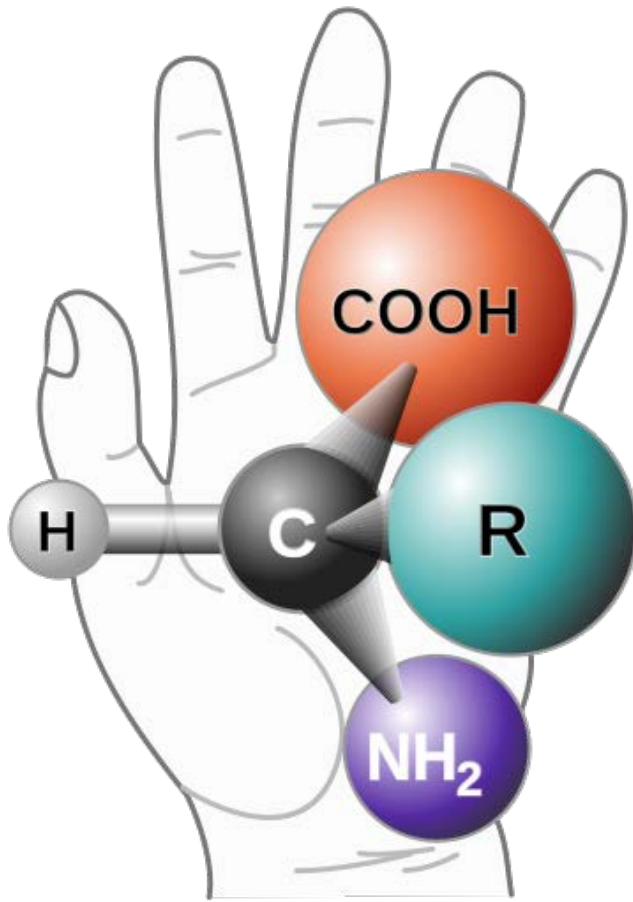


L- (S-) Alanin

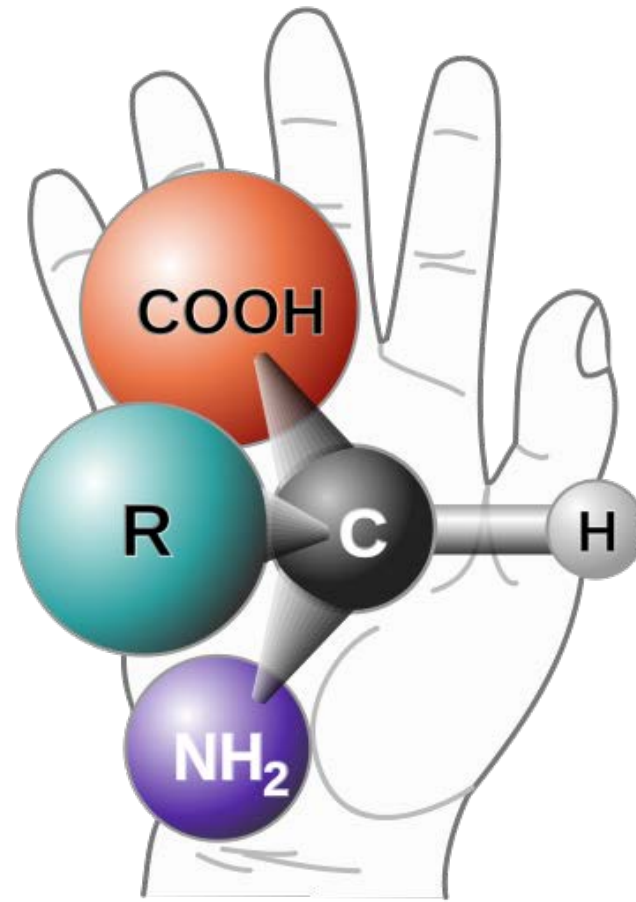


D- (R-) Alanin

Die zwei chiralen Formen von Alanin

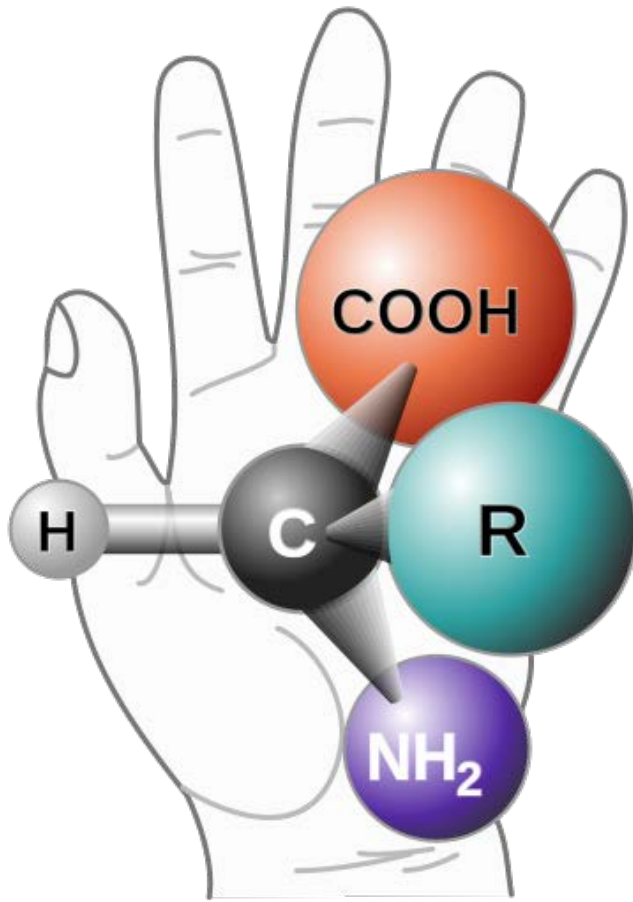


L-Aminosäure



D-Aminosäure

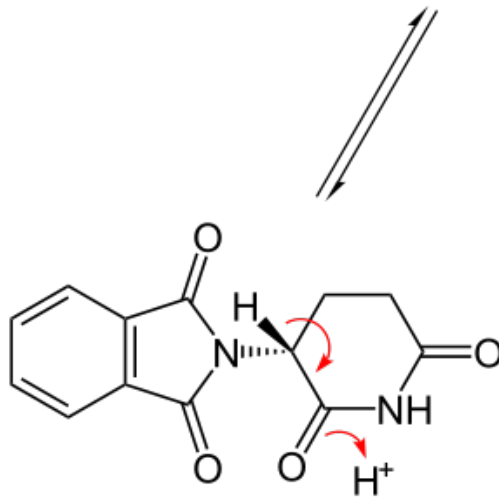
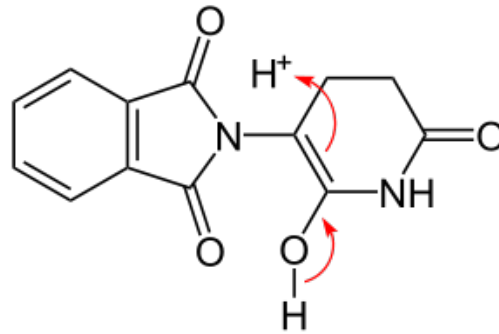
Chiralität der Biomoleküle



L-Aminosäure

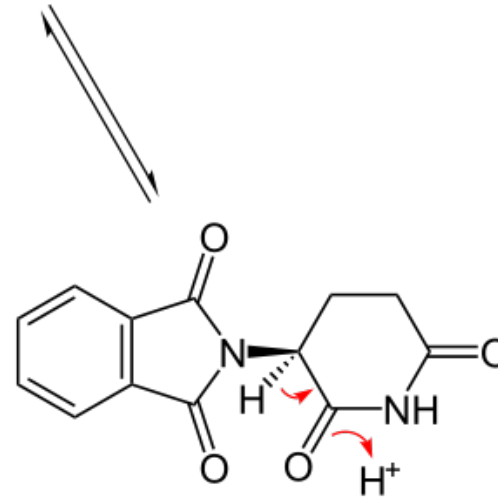
Chiralität der Biomoleküle

achirale tautomere Form



R-Thalidomid

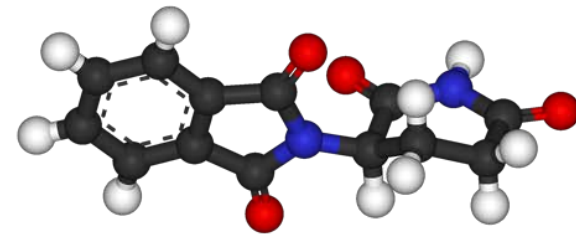
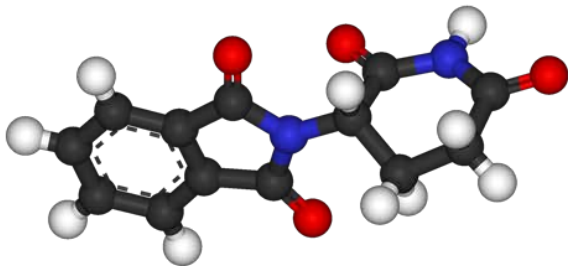
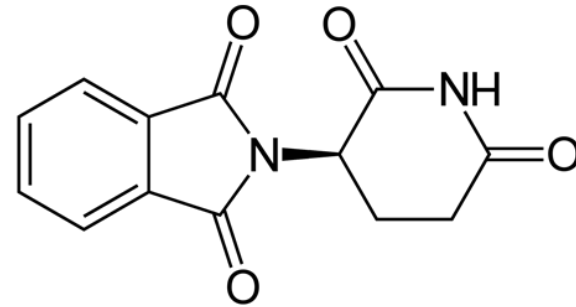
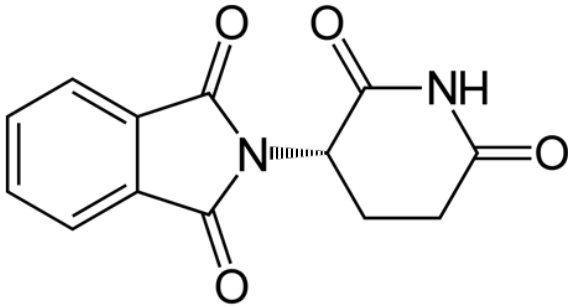
Sedativum



S-Thalidomid

teratogene Wirkung

Thalidomid (Contergan)



Sedativum

teratogene Wirkung

Thalidomid (Contergan)

ON SPONTANEOUS ASYMMETRIC SYNTHESIS

by

F. C. FRANK

The H. H. Wills Physical Laboratory, University of Bristol (England)

I am informed by my colleague Professor W. MOORE that there is still widely believed to be a problem of explaining the original "asymmetric synthesis" giving rise to the general optical activity of the chemical substances of living matter. I have long supposed that this was no problem on the basis of a supposition that the initial production of life is a rare event. We may take as the defining property of a living entity the ability to reproduce its own kind. Omitting such simple entities as flames, which are included by such a definition, and confining attention to chemical molecules, the complexity of any having this essential property of life is likely to be great enough to make it highly improbable that it has a centre of symmetry. It is likely, in fact, to contain α -amino acids which are necessarily asymmetric. Then, if the production of living molecules is an infrequent process, compared with the rate of multiplication of living molecules, the whole earth is likely to be extensively populated with the progeny of the first before another appears. In fact they may have so modified the environment by then that no other has a chance of generation. There are, of course, variants of this hypothesis: e.g. that a second living molecule is produced before the progeny of the first has colonised the whole earth, and competes successfully with it for nutrient material, "starving", or even "poisoning" the other out of existence. This leads to the same result, and depends essentially on the same initial hypothesis, that spontaneous germination of life is a rare event.

Die theoretische Vorhersage
der Erzeugung von Chiralität
durch autokatalytische
asymmetrische Synthese im
Jahre **1953** durch
Frederick Charles **Frank**



Kenso Soai, 1950 -

Kenso Soai 1995

Michael Mauksch and
Svetlana Tsogoeva 2007Reaktionen mit einem etwas
erweiterten Frank Mechanismus

Asymmetric autocatalysis and amplification of enantiomeric excess of a chiral molecule

Kenso Soai, Takanori Shibata, Hiroshi Morioka
& Kaori Choji

Department of Applied Chemistry, Faculty of Science,
Science University of Tokyo, Kagurazaka, Shinjuku-ku, Tokyo 162,
Japan

THE homochirality of natural amino acids and sugars remains a puzzle for theories of the chemical origin of life¹⁻¹⁸. In 1953 Frank⁷ proposed a reaction scheme by which a combination of autocatalysis and inhibition in a system of replicating chiral molecules can allow small random fluctuations in an initially racemic mixture to tip the balance to yield almost exclusively one enantiomer. Here we show experimentally that autocatalysis in a chemical reaction can indeed enhance a small initial enantiomeric excess of a chiral molecule. When a 5-pyrimidyl alkanol with a small (2%) enantiomeric excess is treated with diisopropylzinc and pyrimidine-5-carboxaldehyde, it undergoes an autocatalytic reaction to generate more of the alkanol. Because the reaction involves a chiral catalyst generated from the initial alkanol, and because the catalytic step is enantioselective, the enantiomeric excess of the product is enhanced. This process provides a mechanism by which a small initial imbalance in chirality can become overwhelming.

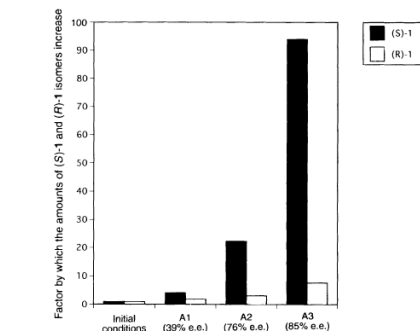


FIG 1. Asymmetric autocatalysis of chiral pyrimidyl alkanol (**1**). Runs A1–3 correspond to Table 1. The enantiomeric excess of (*S*)-**1** increases from 5 to 89% e.e. without the use of additional chiral auxiliaries. During the reactions (runs A1–3), the (*S*)-**1** increases by a factor of 94 times, while (*R*)-**1** increases by a factor of only eight times.

employed as asymmetric autocatalyst, the e.e. of the mixture of catalyst and the product was also 88% (run B5). Thus in series A and B, the low e.e. of (*S*)-**1** was autocatalytically amplified to 88–89%, and the amount of (*S*)-**1** was increased by a factor



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CHIRALITY 19:816–825 (2007)

Demonstration of Spontaneous Chiral Symmetry Breaking in Asymmetric Mannich and Aldol Reactions

MICHAEL MAUKSCH,* SVETLANA B. TSOGOEVA,*[†] SHENGWEI WEI, AND IRINA M. MARTYNOVA
Institute of Organic Chemistry I, University of Erlangen-Nuremberg, Henkestrasse 42, 91052 Erlangen, Germany

ABSTRACT Spontaneous symmetry breaking in reactive systems, known as a rare physical phenomenon and for the Soai autocatalytic irreversible reaction, might in principle also occur in other, more common asymmetric reactions when the chiral product is capable to promote its formation and an element of “nonlinearity” is involved in the reaction scheme. Such phenomena are long sought after in chemistry as a possible explanation for the biological homochirality of biomolecules. We have investigated homogeneous organic stereoselective Mannich and Aldol reactions, in which the product is capable to form H-bridged complexes with the prochiral educt, and found by applying NMR spectroscopy, HPLC analysis, and optical rotation measurements 0.3–50.8% of random product enantiomeric excess under essentially achiral reaction conditions. These findings imply a hitherto overlooked mechanism for spontaneous symmetry breaking and, hence, a novel approach to the problem of absolute asymmetric synthesis and could have also potential significance for the conundrum of homochirality. *Chirality* 19:816–825, 2007. © 2007 Wiley-Liss, Inc.

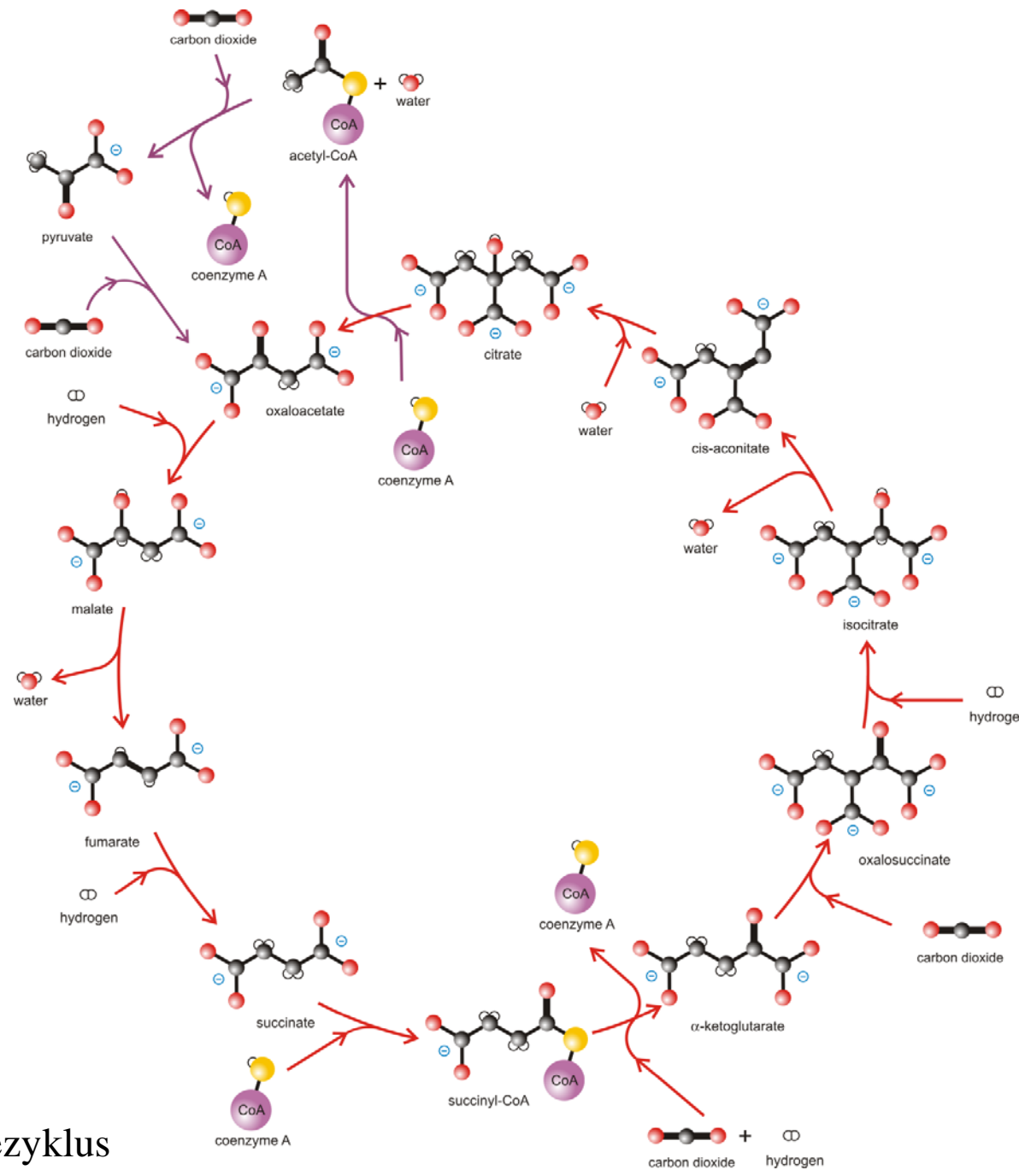
KEY WORDS: organocatalysis; spontaneous symmetry breaking; asymmetric autocatalysis; Mannich reaction; Aldol reaction; homochirality

Einfache Metabolismen und Recycling

Primitiver Metabolismus??



zwölf Teilschritte



G. Wächtershäuser. Before enzymes and templates: Theory of surface metabolism. 1988. *Microbiol. Rev.* **52**:452-484.

Die Umkehrung des Zitronensäurezyklus

On the Crucial Stages in the Origin of Animate Matter

Shneior Lifson

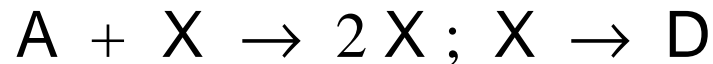
Chemical Physics Department, Weizmann Institute of Science, Rehovot 76100, Israel

Received: 29 March 1996 / Accepted: 30 May 1996

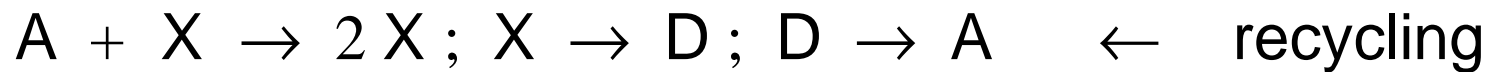
Key words: Origin — Animate matter — Autocatalysis — Natural selection — Sequels — Complexity — Metabolism — Cellular organization — Genetic code



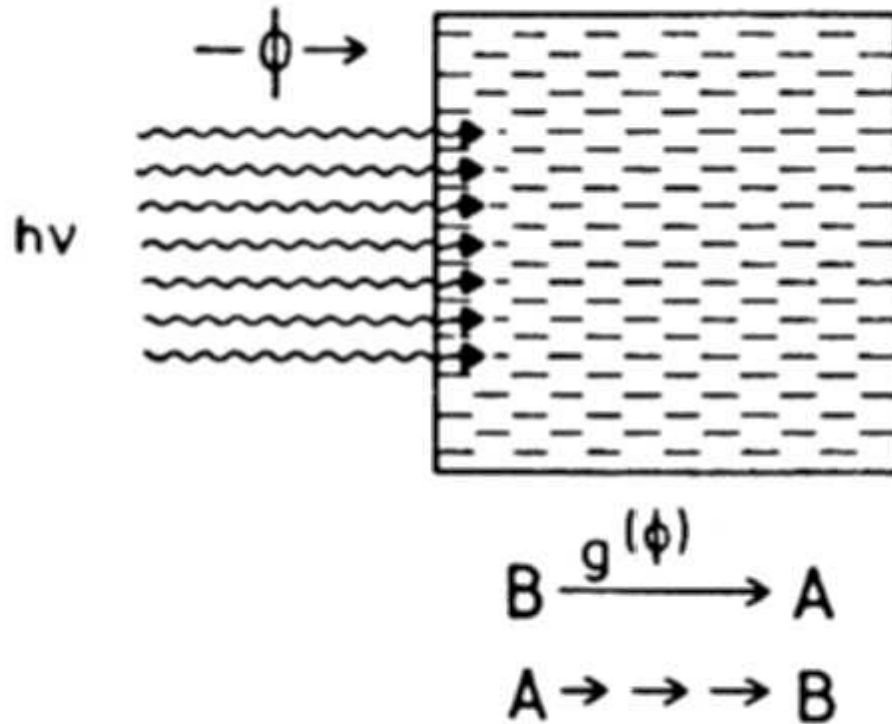
Shneior Lifson, 1914 - 2001



Here, suffice it to recognize that *adaptation of autocatalysts to their changing environment by incorporating sequels into the autocatalytic process yields a great selective advantage.*



Shneior Lifson and recycling in origin of life models



„Los Alamos Bug“

recycling open systems for studying evolution in vitro

Ribonukleinsäuren - RNA-Welt



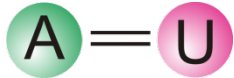
A ≡ Adenine

G ≡ Guanine

U ≡ Uracil

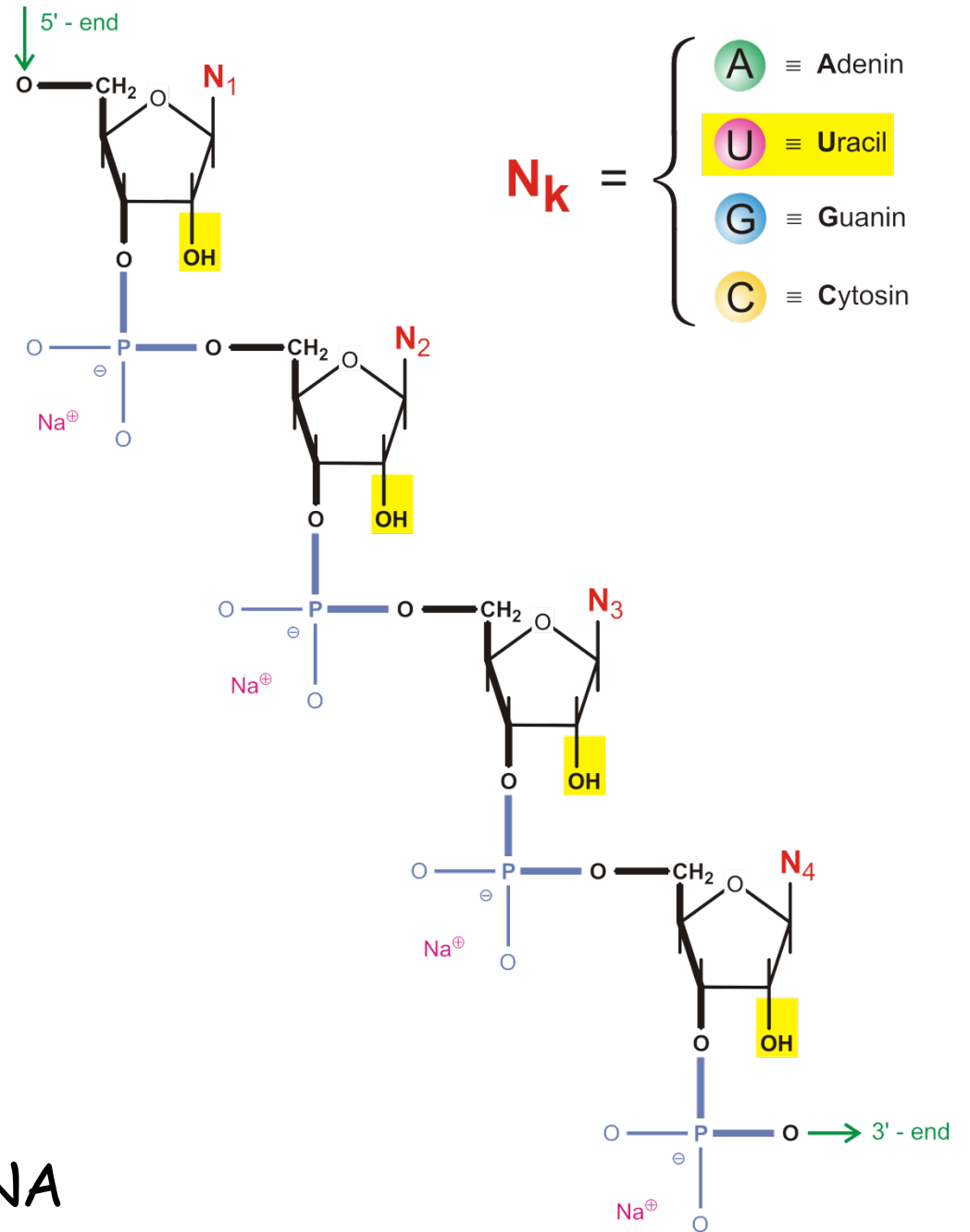
C ≡ Cytosine

Ribonucleinsäure RNA



Nucleotidpaare

Ribonucleinsäure RNA

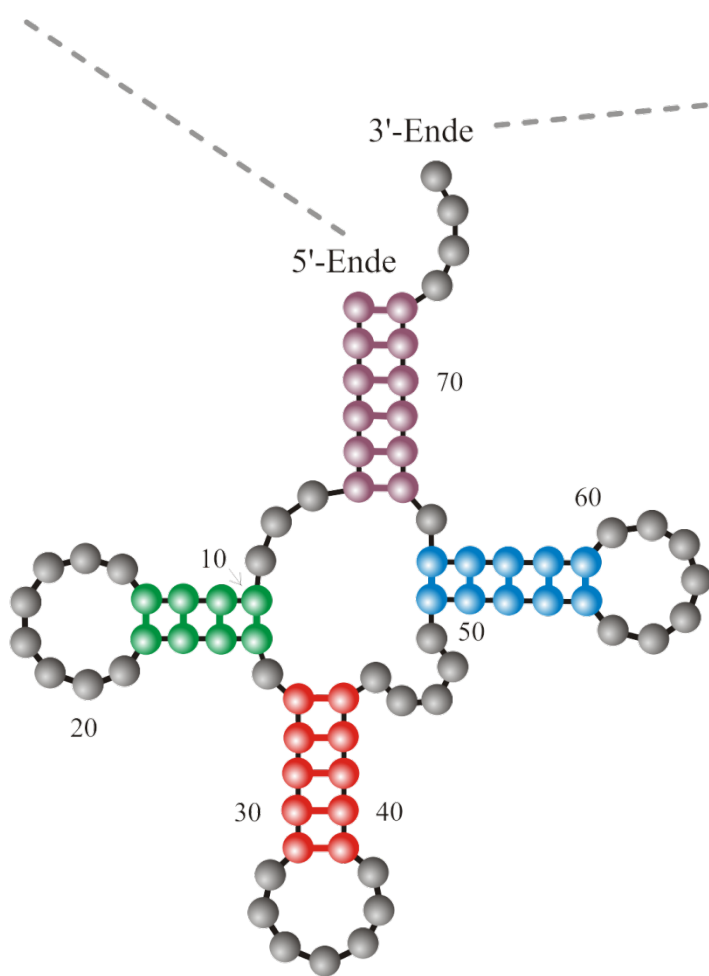


Sequenz

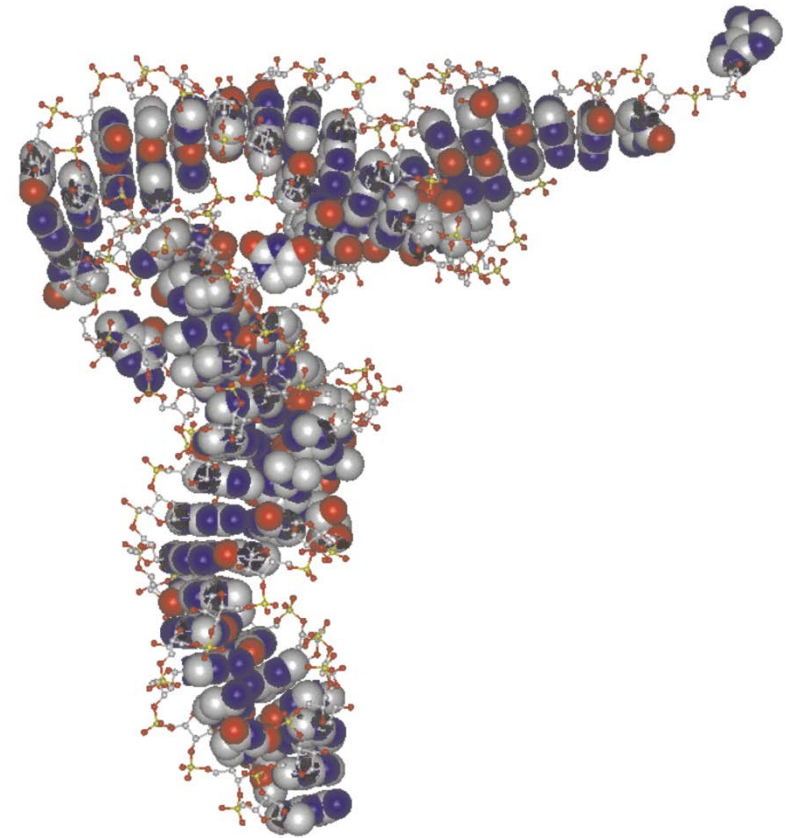
5'-Ende

3'-Ende

GCGGAUUUAGCUCAGDDGGGAGAGCMCCAGACUGAAYAUCUGGAGMUC CUGUGTPCGAUC CACAGAAUUCGCACCA



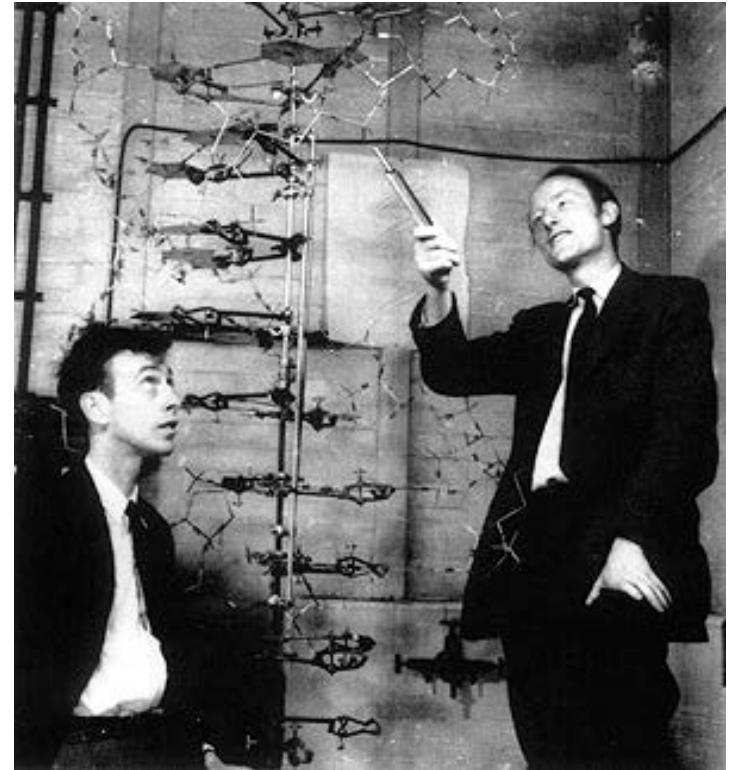
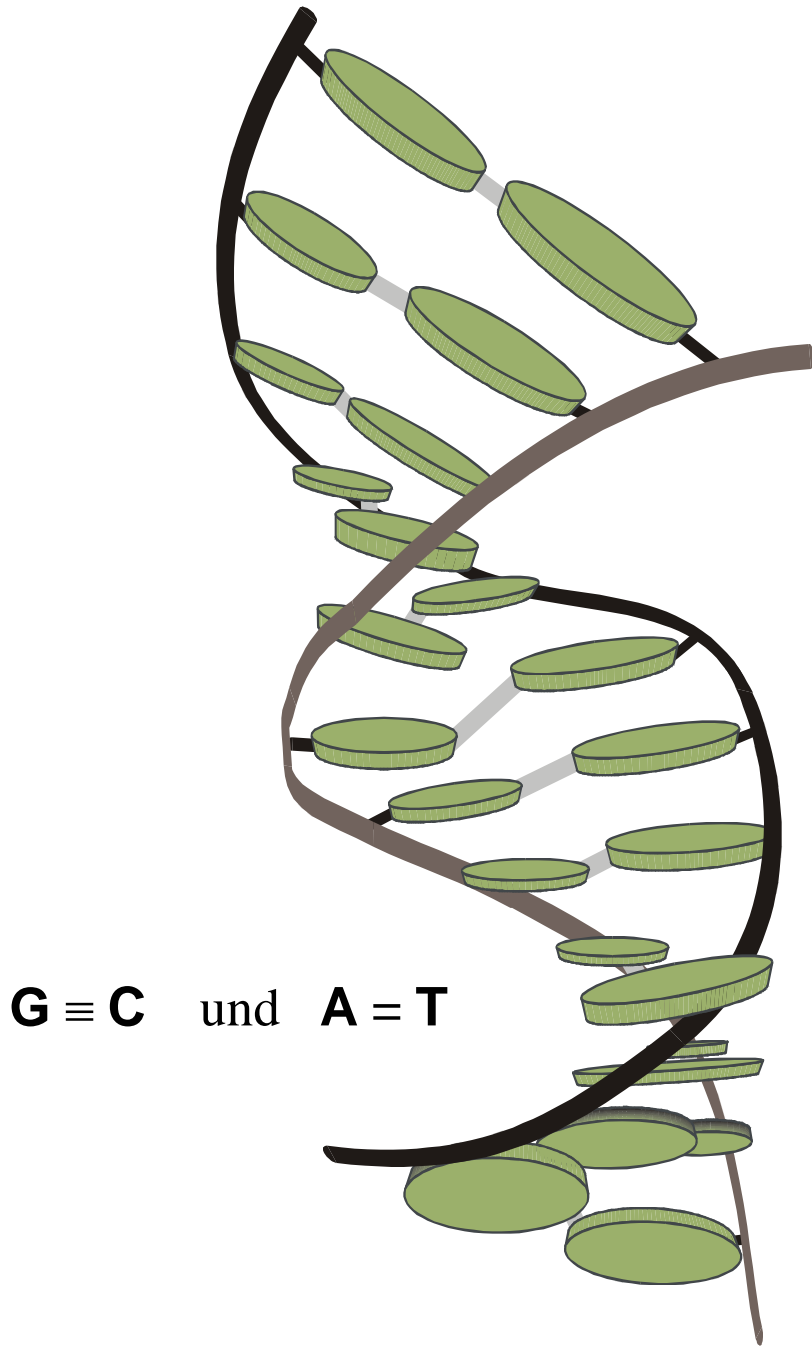
Sekundärstruktur



räumliche Struktur

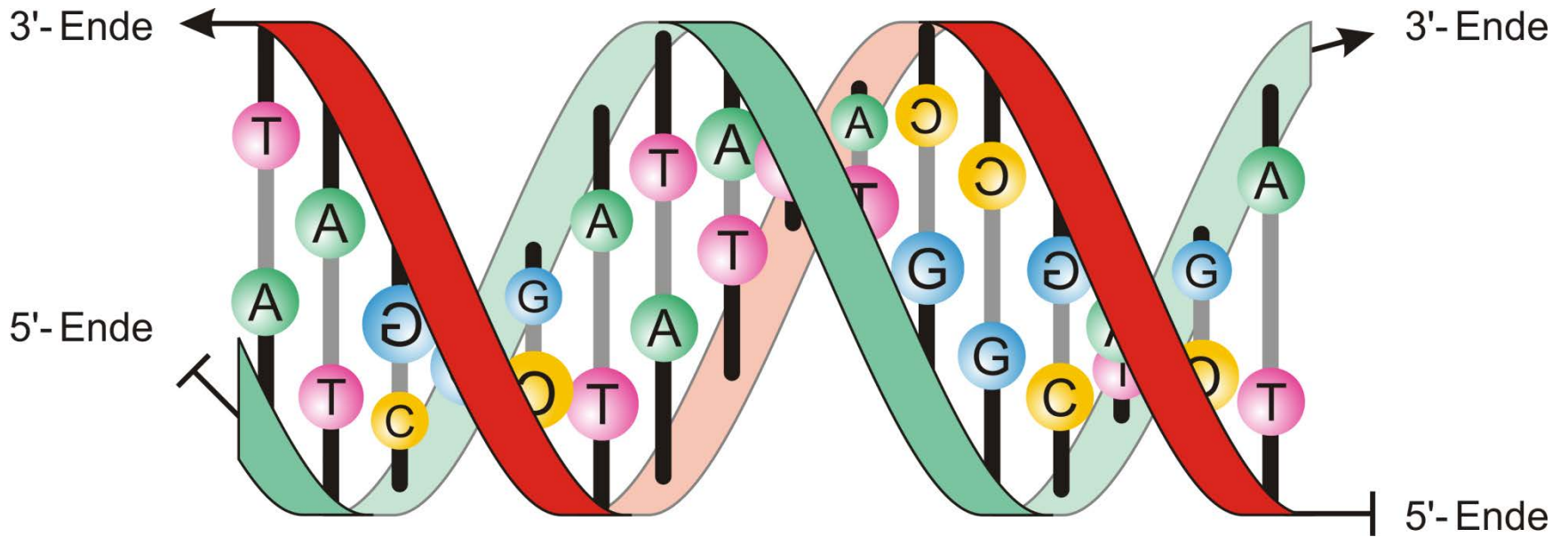
Ribonucleinsäure RNA

Desoxyribonukleinsäuren und Proteine

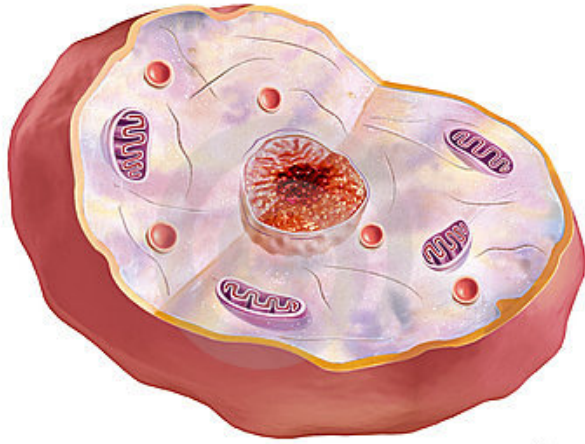


James D. Watson, 1928- , and Francis Crick, 1916-2004,
Nobel Preis 1962

Die dreidimensionale Struktur eines
kleinen Stückes der B-DNA



Die B-Form der DNA-Doppelhelix



Zelldurchmesser

0,01 mm



DNA-Länge

1 m

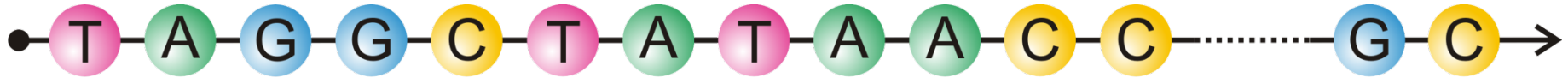
1 m

Vergrößerung 1 : 100 000

100 km

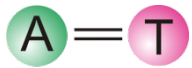
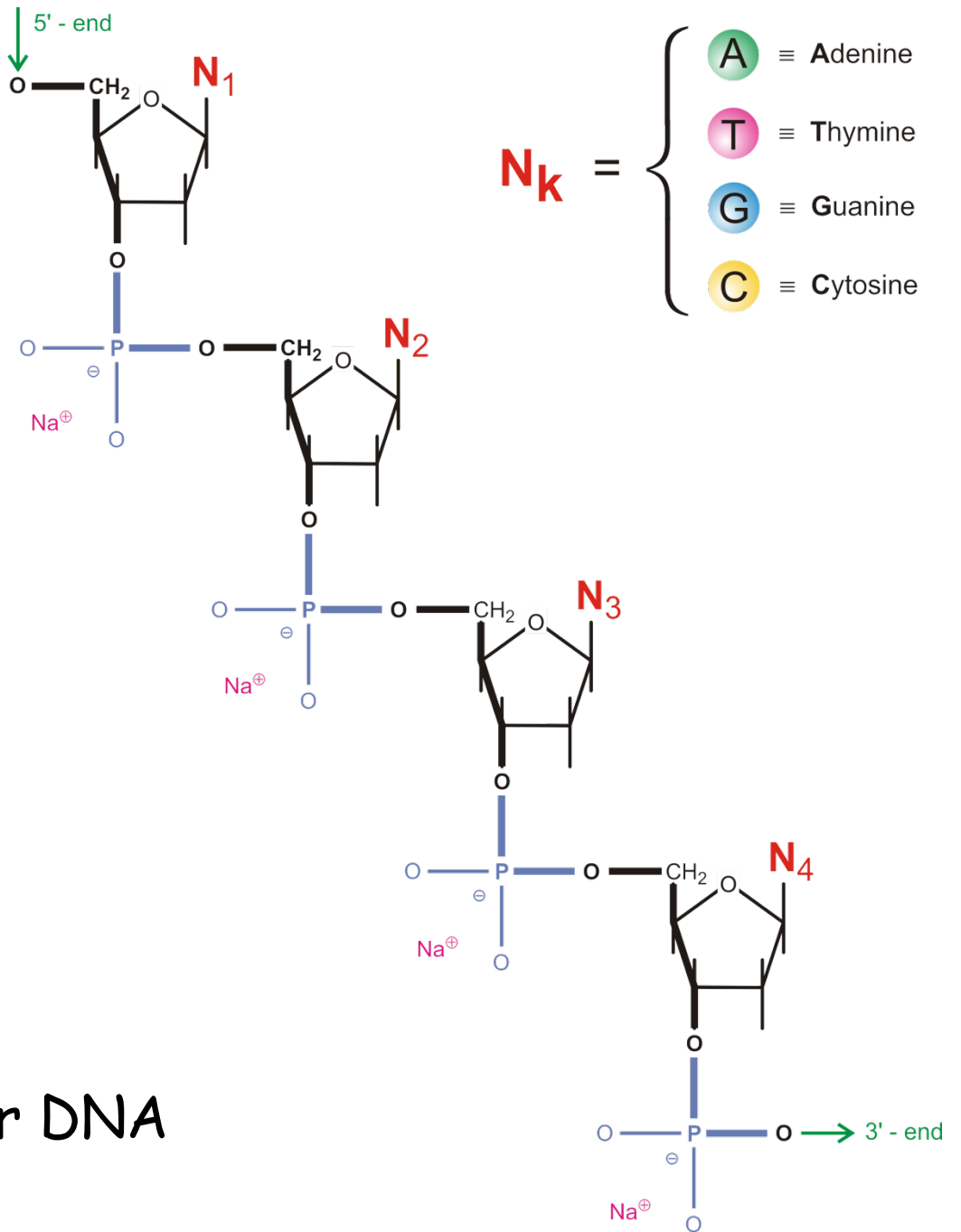
die menschliche DNA ist 3 Milliarden Basenpaare lang

Größenverhältnisse



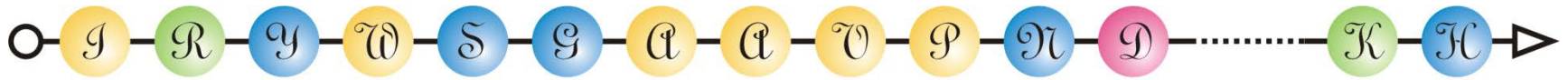
A ≡ Adenin T ≡ Thymin G ≡ Guanin C ≡ Cytosin

Desoxyribonucleinsäure DNA



Nucleotidpaare

Chemische Formel der DNA



A ≡ **alanine**

G ≡ **glycine**

M ≡ **methionine**

S ≡ **serine**

C ≡ **cysteine**

H ≡ **histidine**

N ≡ **asparagine**

T ≡ **threonine**

D ≡ **aspartic acid**

I ≡ **isoleucine**

P ≡ **proline**

V ≡ **valine**

E ≡ **glutamic acid**

K ≡ **lysine**

Q ≡ **glutamine**

W ≡ **tryptophane**

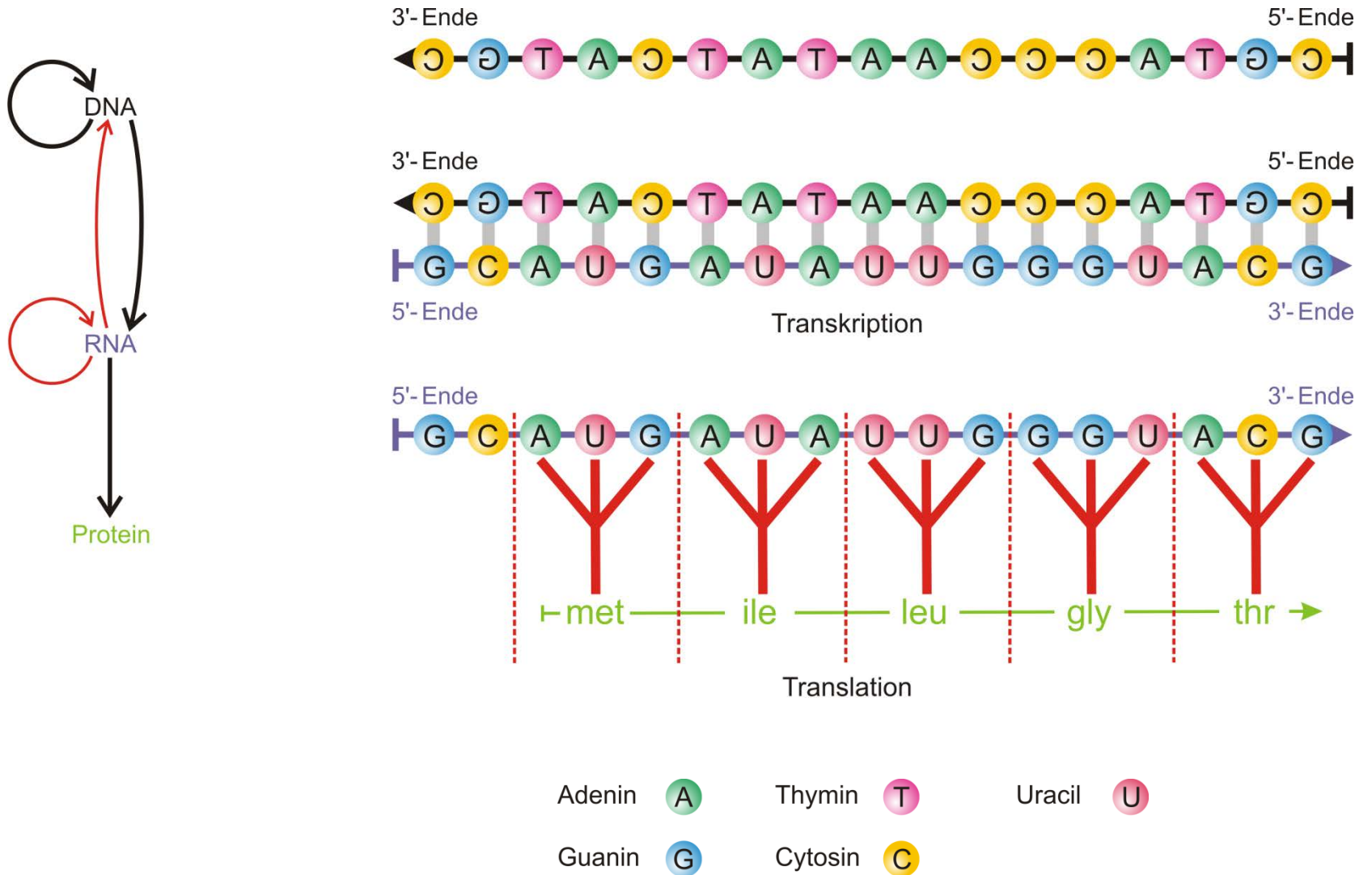
F ≡ **phenyl alanine**

L ≡ **leucine**

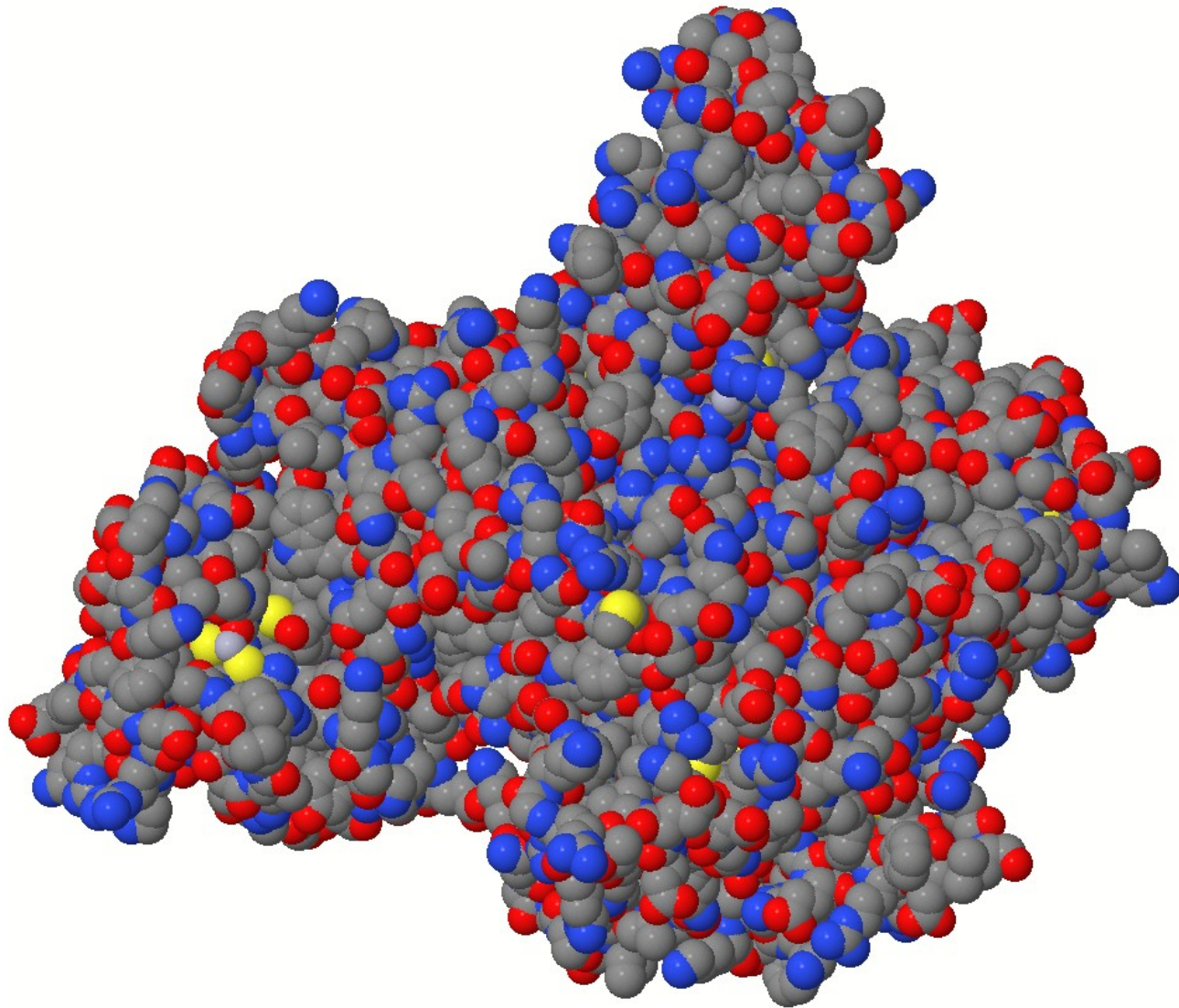
R ≡ **arginine**

Y ≡ **tyrosine**

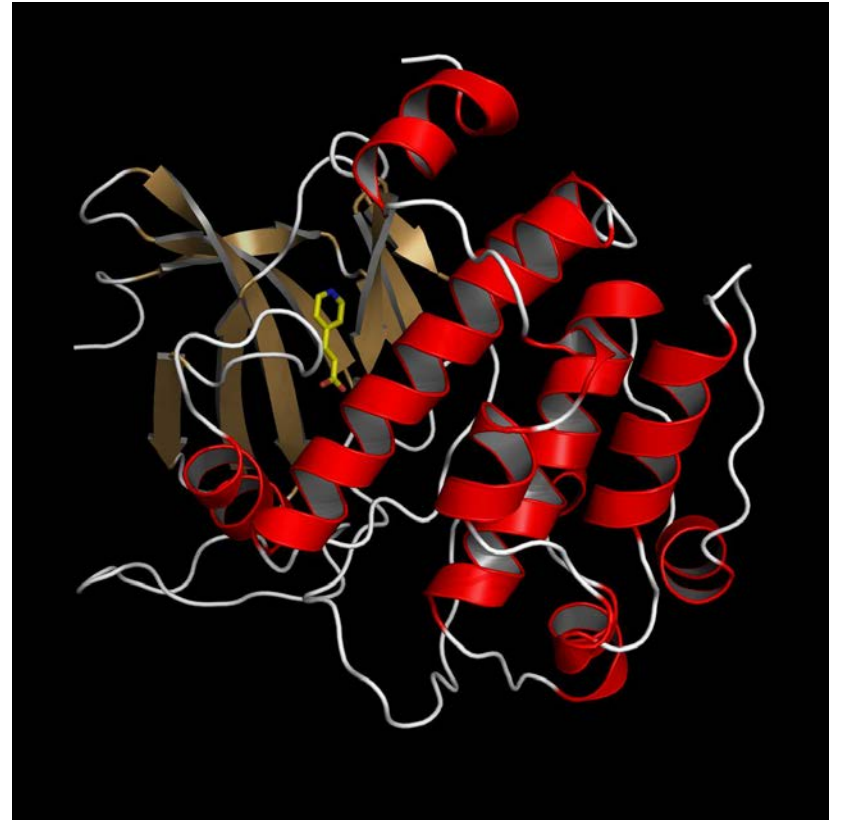
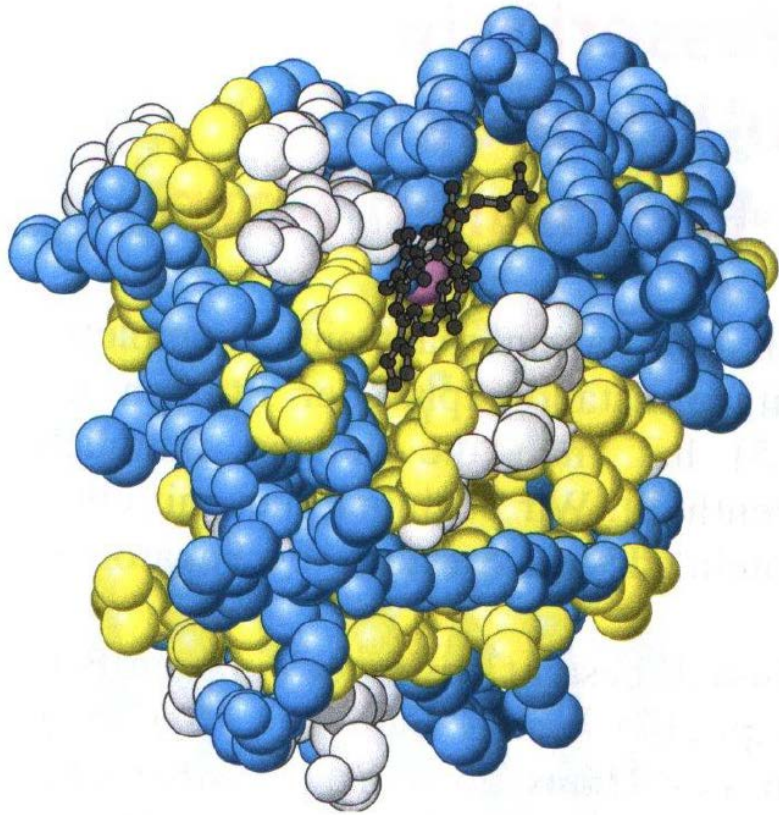
Protein



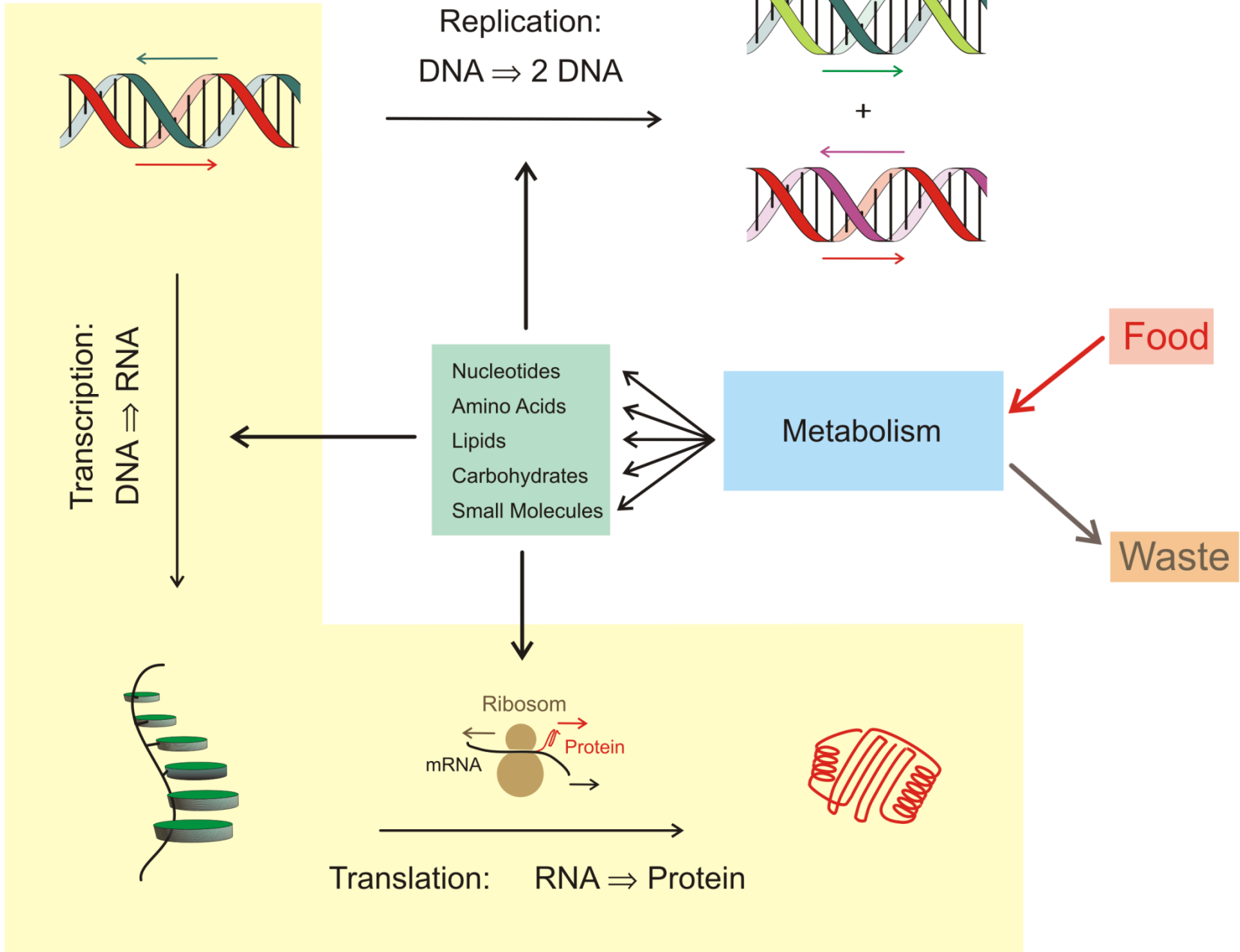
The ,central dogma' of molecular biology



Transkriptionsenzym: DNA \rightarrow RNA



Proteinstrukturen

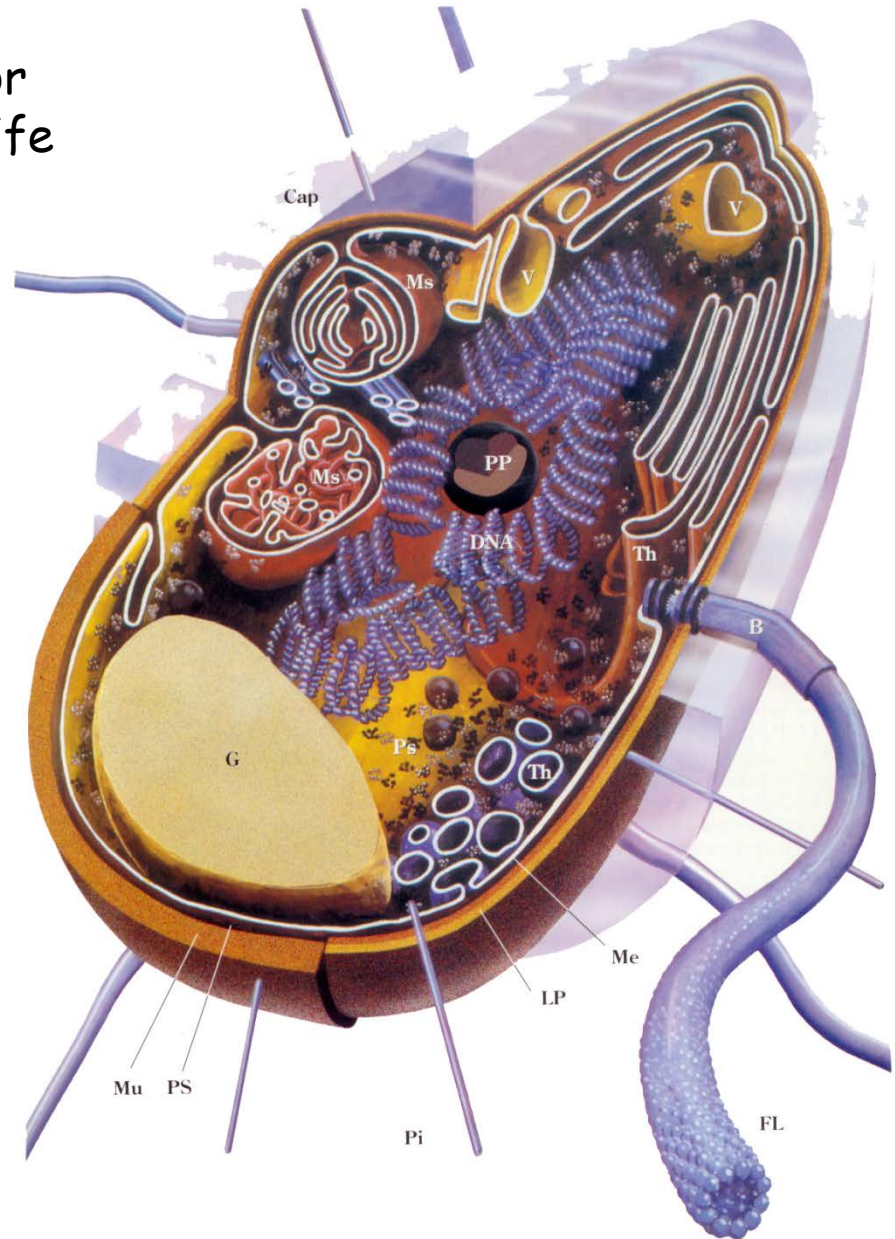


The bacterial cell as an example for the simplest form of autonomous life

Escherichia coli genome:

4 million nucleotides

4460 genes



The spatial structure of the bacterium *Escherichia coli*

Evolution: Das Prinzip der Biologie

1. Prolog
2. Darwinsche Selektion so einfach wie möglich
3. Variation I: Rekombination
4. Variation II: Mutation
5. Von der Theorie zur Anwendung
6. Evolution zu höherer Komplexität
7. Viroide, Virusspezies und Bakterien
8. Epilog

Prolog

Nothing in biology makes sense
except in the light of evolution.



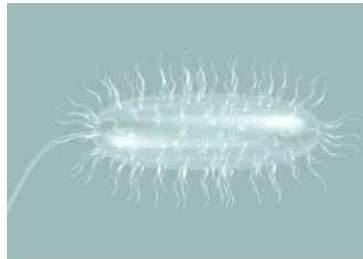
Theodosius Dobzhansky,
1900 – 1975



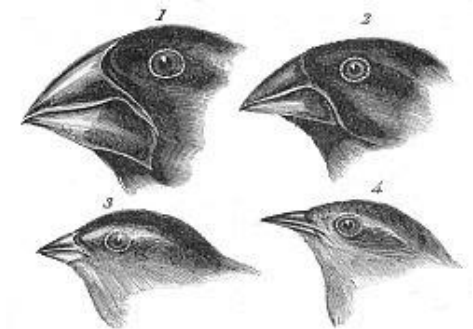
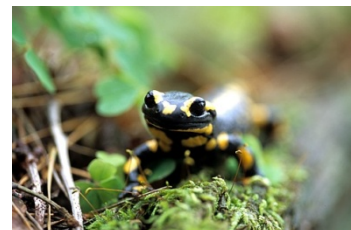
Charles Darwin, 1809 - 1882



Voyage on HMS Beagle, 1831 - 1836



Phänotypen



1. *Geospiza magnirostris*
2. *Geospiza fortis*
3. *Geospiza parvula*
4. *Certhidea olivacea*

Finches from Galapagos Archipelago



ON
THE ORIGIN OF SPECIES

BY MEANS OF NATURAL SELECTION,

OR THE

PRESERVATION OF FAVOURED RACES IN THE STRUGGLE
FOR LIFE.

By CHARLES DARWIN, M.A.,

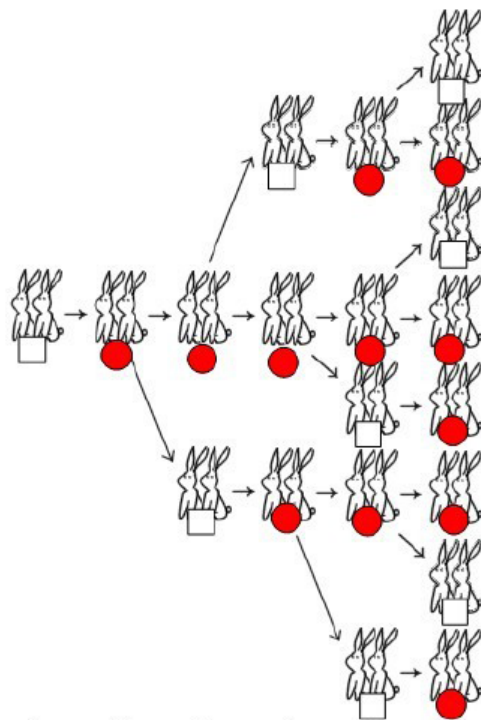
FELLOW OF THE ROYAL, GEOLOGICAL, LINNEAN, ETC., SOCIETIES;
AUTHOR OF 'JOURNAL OF RESEARCHES DURING H. M. S. BEAGLE'S VOYAGE
ROUND THE WORLD.'

LONDON:
JOHN MURRAY, ALBEMARLE STREET.

1859.

The right of Translation is reserved.

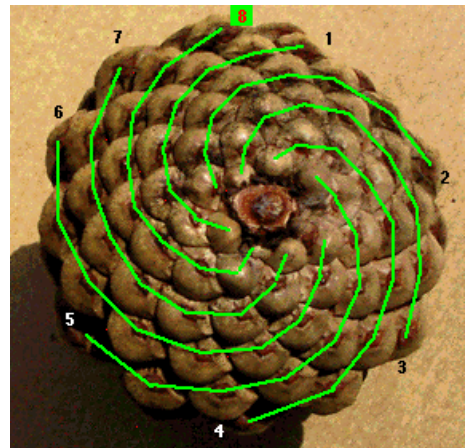
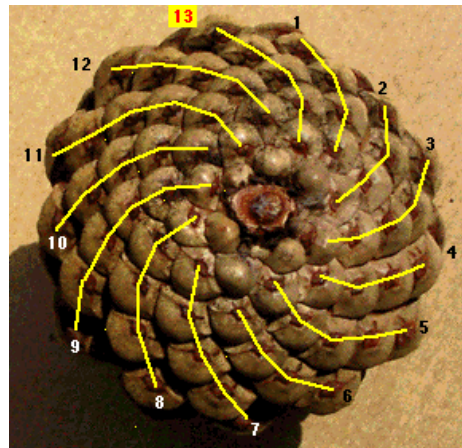
$$F_{n+1} = F_n + F_{n-1}; F_0 = 0, F_1 = 1$$



pairs = 1	1	2	3	5	8
generation = 1	2	3	4	5	6



Leonardo da Pisa
 „Fibonacci“
 ~1180 – ~1240





Thomas Robert Malthus
1766 – 1834

Wachstum tierisch-menschlicher Populationen
führt auf eine geometrische Reihe:

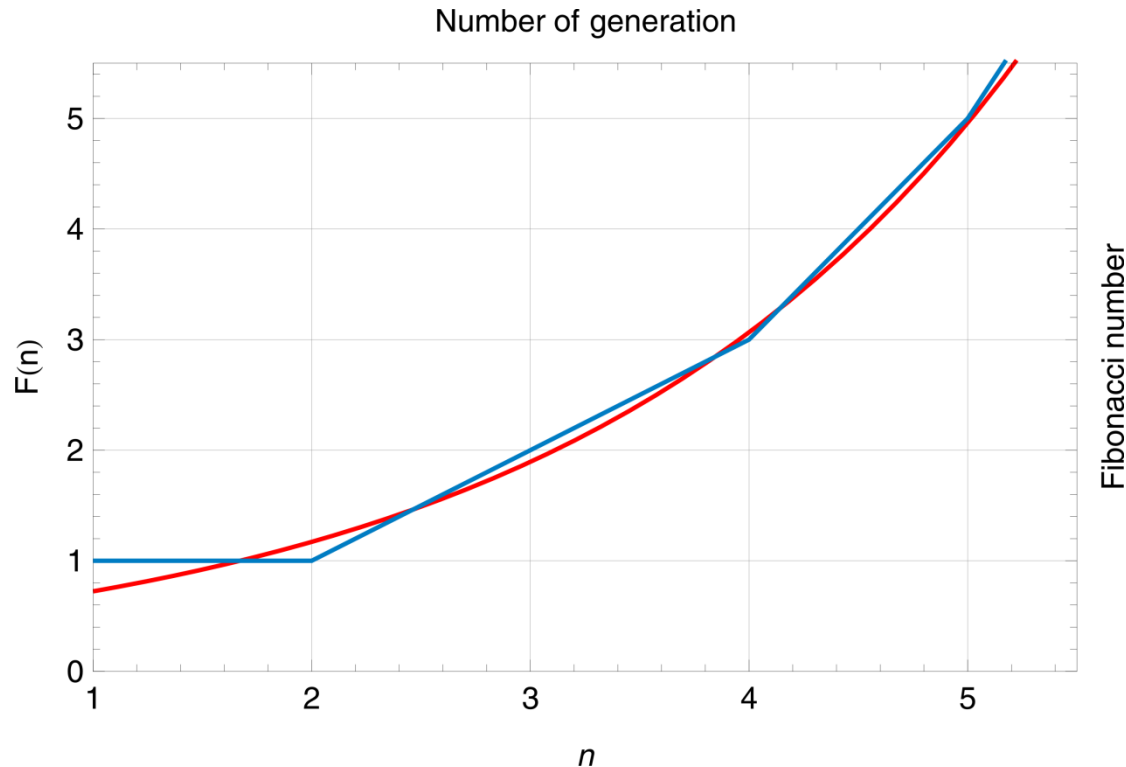
$2 \rightarrow 4 \rightarrow 8 \rightarrow 16 \rightarrow 32 \rightarrow 64 \rightarrow 128 \rightarrow 256 \rightarrow$

$$\frac{dN}{dt} = r N(t), \quad N(t) = N_0 \exp(rt)$$

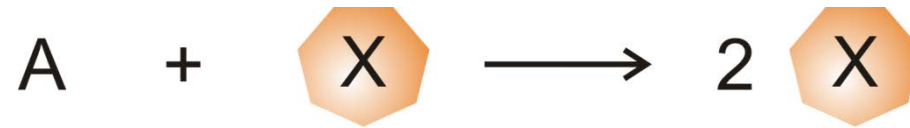
Exponentialfunktion



Leonhard Euler
1707 – 1783



autocatalysis



$$\frac{dx}{dt} = f x \Rightarrow x(t) = x(0) \exp(ft)$$



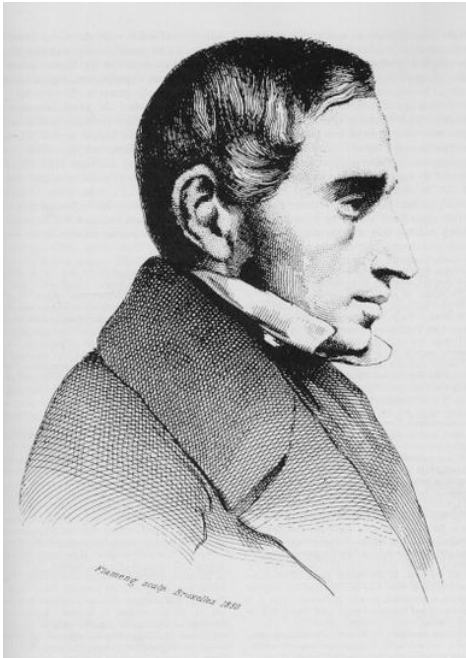
competition

$$\frac{dx_k}{dt} = f_k x_k ; k = 1, 2, \dots, n$$

$$x_k(t) = x_k(0) \exp(f_k t)$$

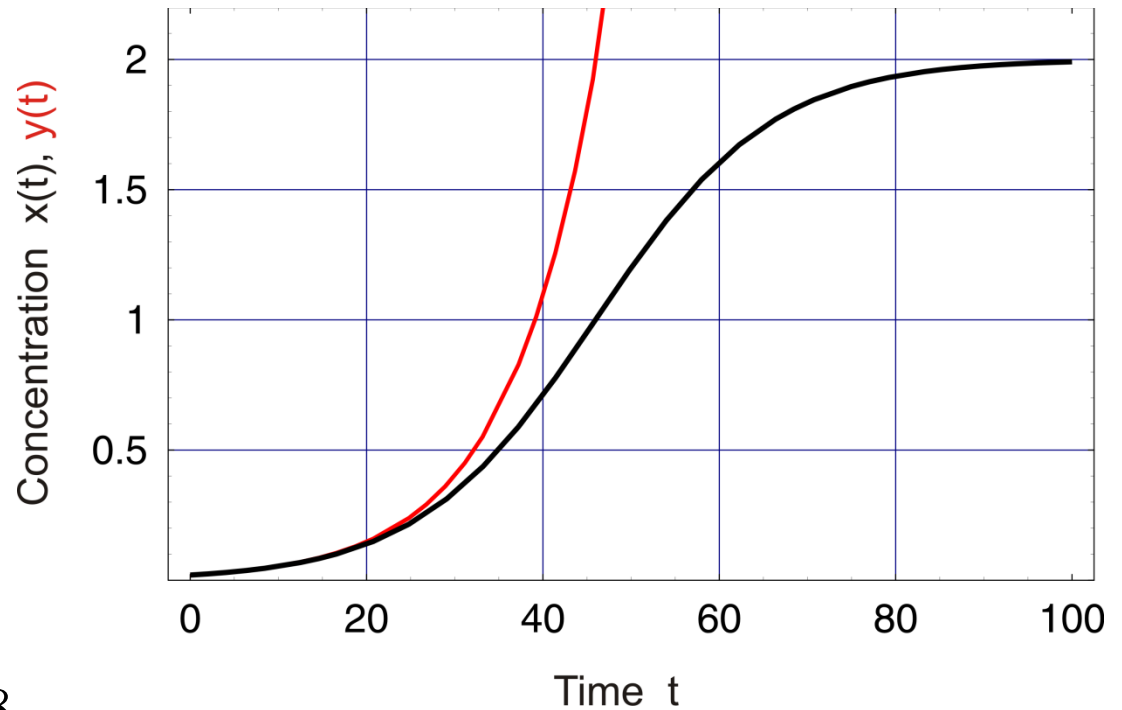
The chemistry and the mathematics of reproduction

Darwinsche Selektion so einfach
wie möglich



Pierre-François Verhulst,
1804-1849

$$\frac{dx}{dt} = r x \left(1 - \frac{x}{C} \right), \quad x(t) = \frac{x(0) C}{x(0) + (C - x(0)) e^{-rt}}$$



The logistic equation, 1828

$$\frac{dx}{dt} = r x \left(1 - \frac{x}{C} \right) \Rightarrow \frac{dx}{dt} = r x - \frac{x}{C} r x$$

$$r x \equiv \Phi(t), C = 1: \frac{dx}{dt} = x(r - \Phi)$$

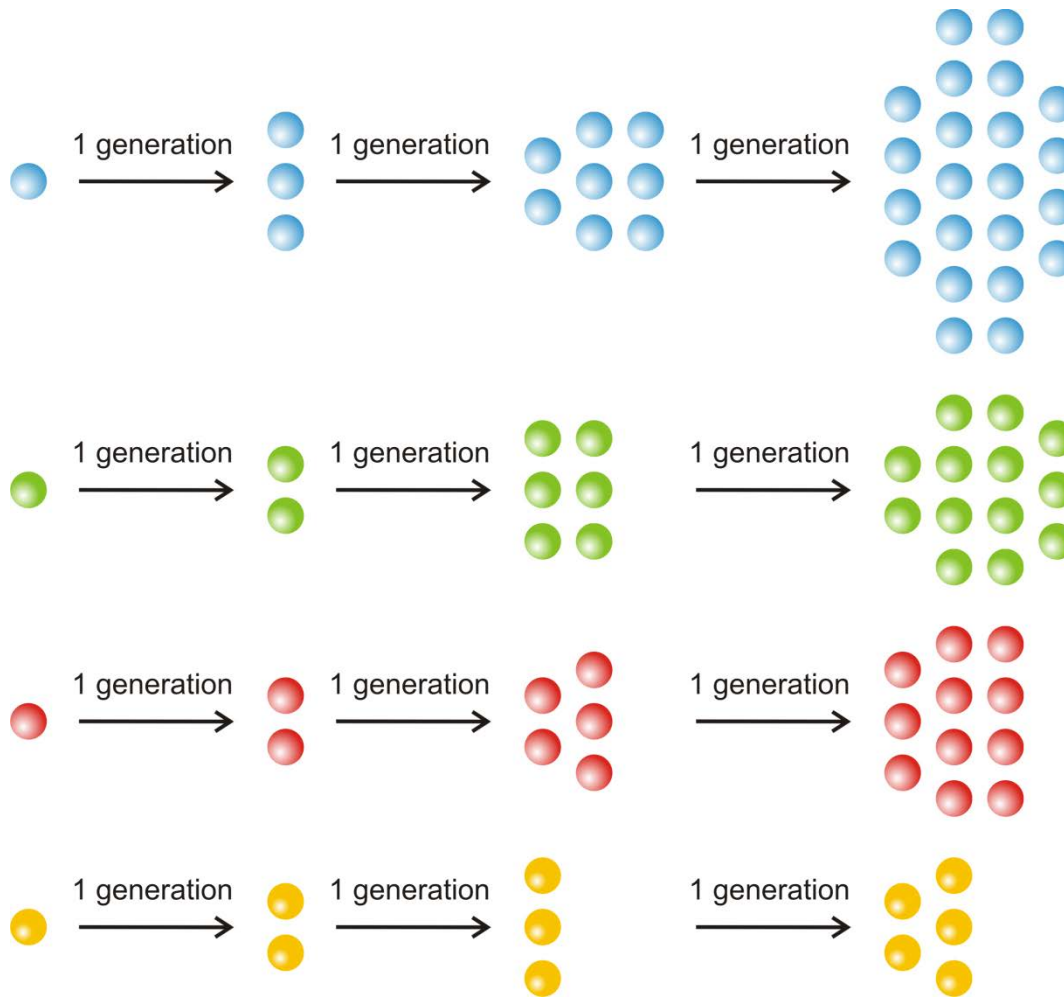
$$X_1, X_2, \dots, X_n: [X_i] = x_i; \sum_{i=1}^n x_i = C = 1$$

$$\frac{dx_j}{dt} = x_j \left(f_j - \sum_{i=1}^n f_i x_i \right) = x_j (f_j - \Phi); \quad \Phi = \sum_{i=1}^n f_i x_i$$

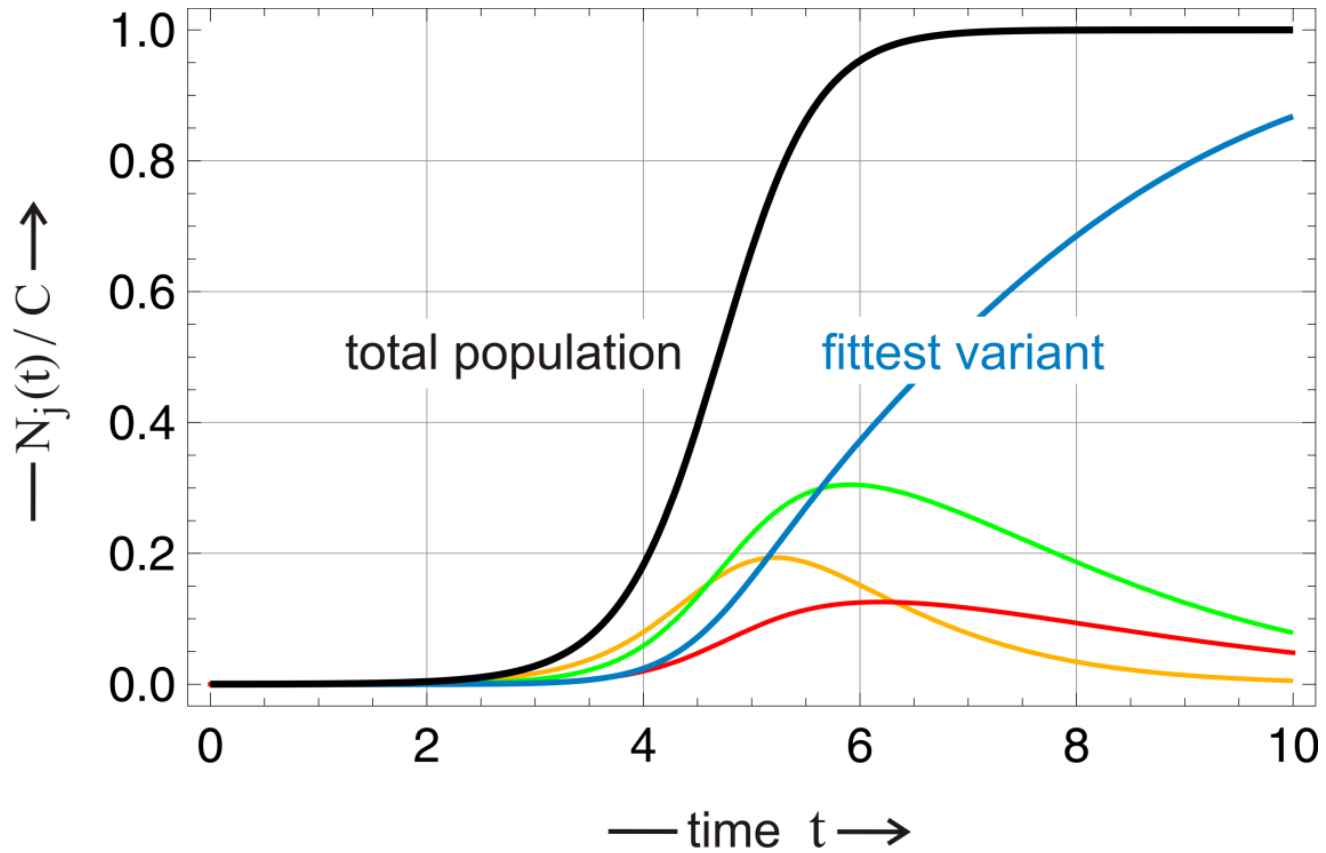
Darwin

$$\frac{d\Phi}{dt} = 2(\langle f^2 \rangle - \langle \bar{f} \rangle^2) = 2 \text{var}\{f\} \geq 0$$

Generalization of the logistic equation to n variables yields selection



fitness values: $f_1 = 2.80$, $f_2 = 2.35$, $f_3 = 2.25$, and $f_4 = 1.75$



fitness values: $f_1 = 2.80$, $f_2 = 2.35$, $f_3 = 2.25$, and $f_4 = 1.75$



Drei notwendige Bedingungen für Darwinsche Evolution sind:

1. **Vermehrung** (und Vererbung),
2. **Variation**, und
3. **Selektion**.

Vermehrung führt zu exponentiellem Wachstum, das eine *conditio sine qua non* für Selektion darstellt.

Variation ist ein Nebeneffekt des molekularen Mechanismus der Reproduktion.

Selektion ist eine Konsequenz der endlichen Ressourcen.

Da im Sinne der Optimierung von Fitness durch die Darwinsche Evolution nur Nachkommen gezählt werden, ist sie fast universell gültig.

Darwins principle is still valid but **nothing**
he said about **inheritance**, multiplication
and **variation** is correct.

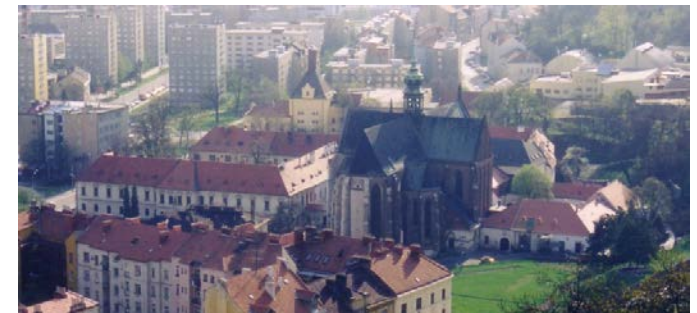
Variation I: Gregor Mendels Genetik und Rekombination

1. flower color is purple or white
2. flower position is axil or terminal
3. stem length is long or short
4. seed shape is round or wrinkled
5. seed color is yellow or green
6. pod shape is inflated or constricted
7. pod color is yellow or green

1st experiment ⇒ 60 fertilizations on 15 plants
 2nd experiment ⇒ 58 fertilizations on 10 plants
 3rd experiment ⇒ 35 fertilizations on 10 plants
 4th experiment ⇒ 40 fertilizations on 10 plants
 5th experiment ⇒ 23 fertilizations on 5 plants
 6th experiment ⇒ 34 fertilizations on 10 plants
 7th experiment ⇒ 37 fertilizations on 10 plants



Gregor Mendel (1822-1884)



Gregor Mendel's experiments on plant genetics

Versuche über Pflanzen-Hybriden. *Verhandlungen des naturforschenden Vereines in Brünn* **4**: 3–47, 1866.

Über einige aus künstlicher Befruchtung gewonnenen Hieracium-Bastarde. *Verhandlungen des naturforschenden Vereines in Brünn* **8**: 26–31, 1870.

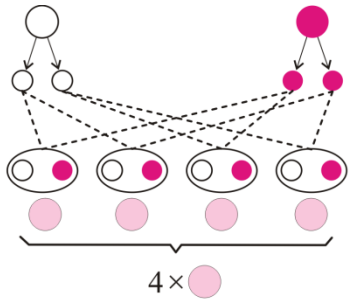
Experiment 1			Experiment 2	
Form of Seed			Color of Albumen	
Plants	Round	Angular	Yellow	Green
1	45	12	25	11
2	27	8	32	7
3	24	7	14	5
4	19	10	70	27
5	32	11	24	13
6	26	6	20	6
7	88	24	32	13
8	22	10	44	9
9	28	6	50	14
10	25	7	44	18

- Expt. 1: Form of seed. From 253 hybrids 7324 seeds were obtained in the second trial year. Among them were 5474 round or roundish ones and 1850 angular wrinkled ones. Therefrom the ratio **2.96:1** is deduced.
- Expt. 2: Color of albumen.. 258 plants yielded 8023 seeds, 6022 yellow, and 2001 green; their ratio, therefore, is as **3.01:1**.

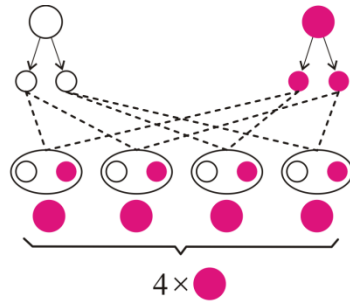
Gregor Mendel concluded correctly from his experiments:

1. that the inheritance of each trait is determined by "units" or "factors" that are passed on to descendents unchanged (these units are now called genes)
2. that an individual inherits one such unit from each parent for each trait
3. that a trait may not show up in an individual but can still be passed on to the next generation.

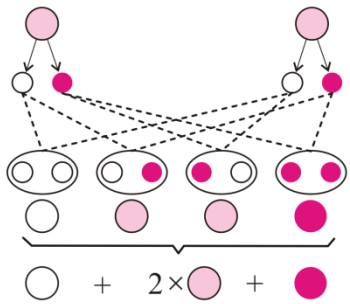
Gregor Mendel's experiments on plant genetics



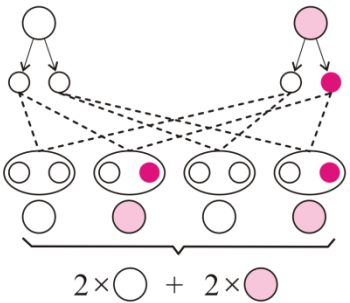
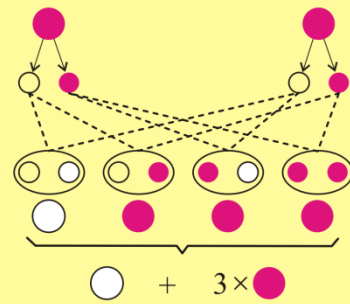
F1



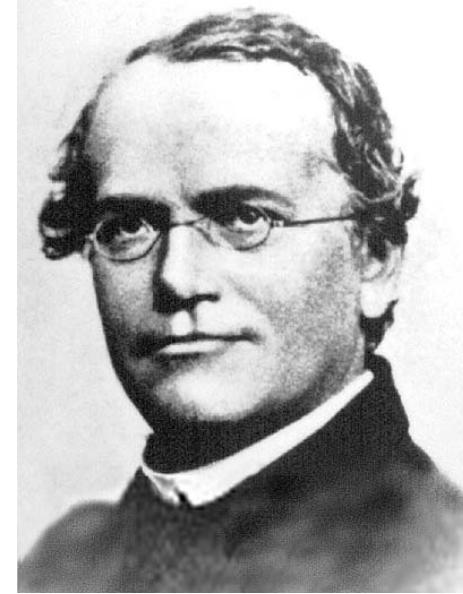
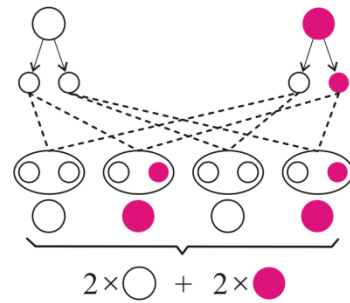
F2



F2



F1 x F2

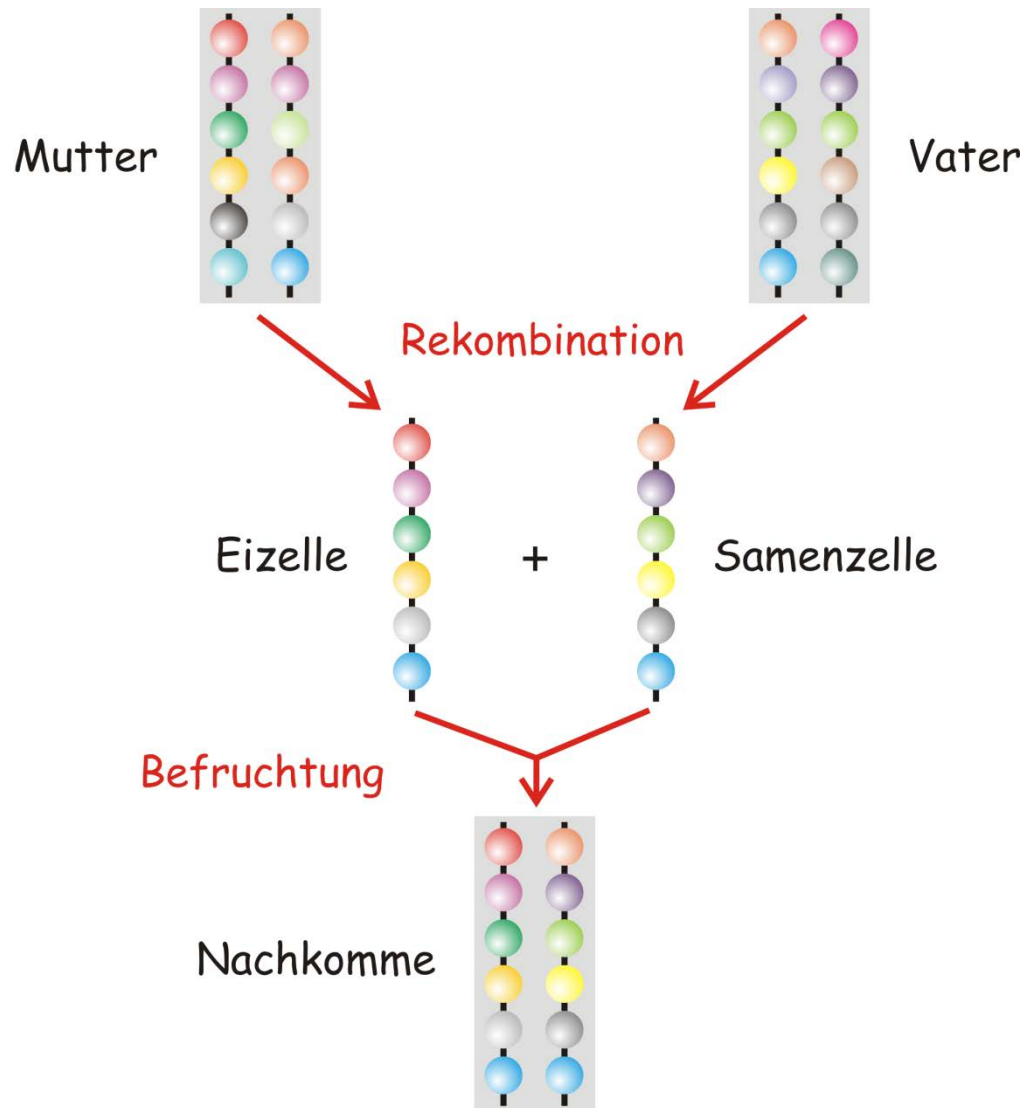


Gregor Mendel, 1822-1884

Intermediäres Allelpaar

Dominant/rezessives Allelpaar

Mendel's rules of inheritance:
white and red colors of flowers



Rekombination in Mendels Genetik



Ronald Fisher (1890-1962)

Allele: A_1, A_2, \dots, A_n

Häufigkeiten: $x_i = [A_i]$; Genotypen: $A_i \cdot A_j$

Fitnesswerte: $a_{ij} = f(A_i \cdot A_j), a_{ij} = a_{ji}$

Mendel

Darwin

$$\frac{dx_j}{dt} = \sum_{i=1}^n a_{ji} x_i x_j - \Phi x_j = x_j \left(\sum_{i=1}^n a_{ji} x_i - \Phi \right), \quad j=1, 2, \dots, n$$

$$\text{mit } \Phi(t) = \sum_{j=1}^n \sum_{i=1}^n a_{ji} x_i x_j \quad \text{und} \quad \sum_{j=1}^n x_j = 1$$

$$\frac{d\Phi}{dt} = 2(\langle \bar{a}^2 \rangle - \langle \bar{a} \rangle^2) = 2 \text{ var}\{\bar{a}\} \geq 0$$

Ronald Fisher's Selektionsgleichung: The genetical theory of natural selection.
Oxford, UK, Clarendon Press, 1930.



Ronald Fisher (1890-1962)



J. B. S. Haldane (1892-1964)



Sewall Wright (1889-1988)

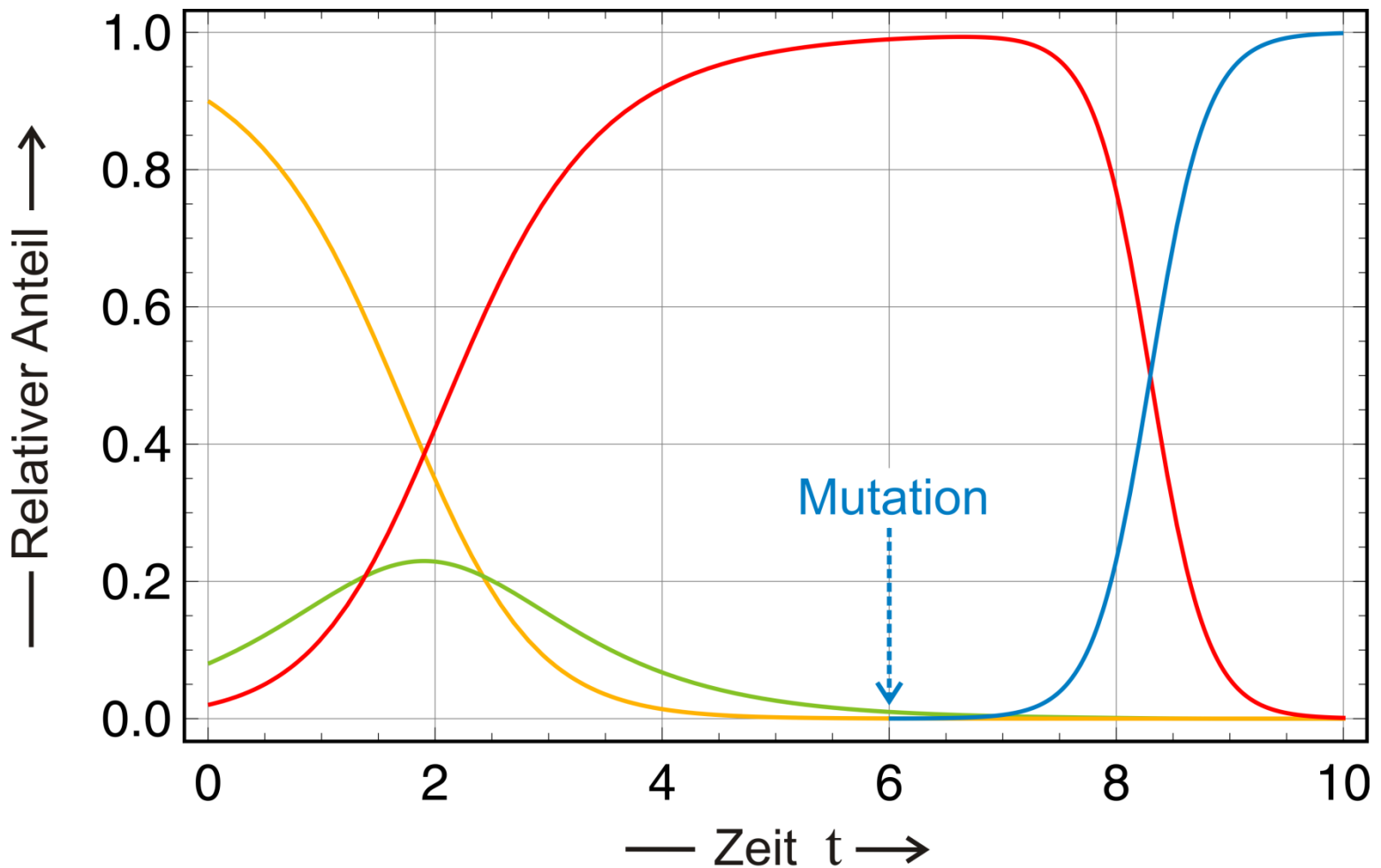
Die drei Begründer der Populationsgenetik



Theodosius Dobzhansky,
1900 – 1975

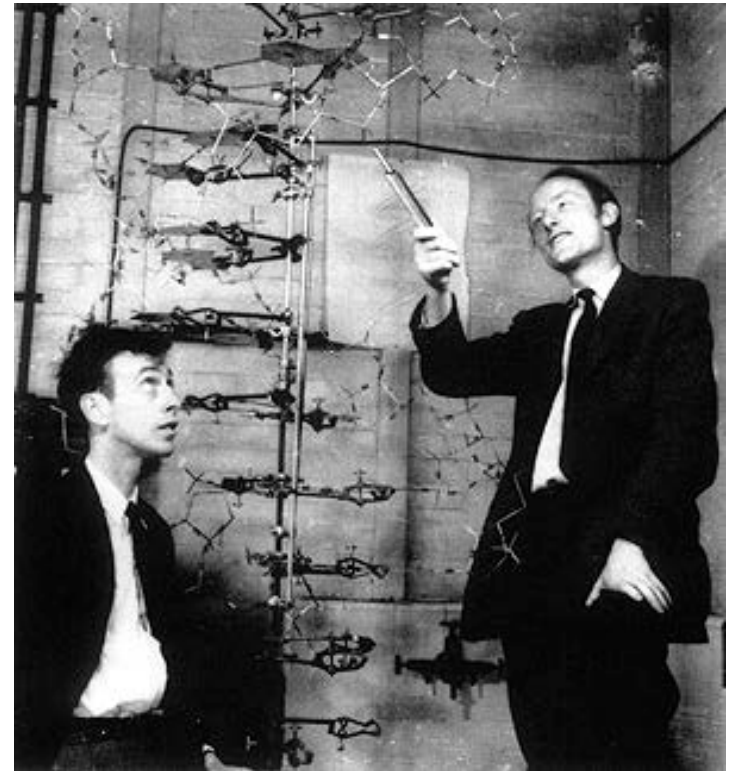
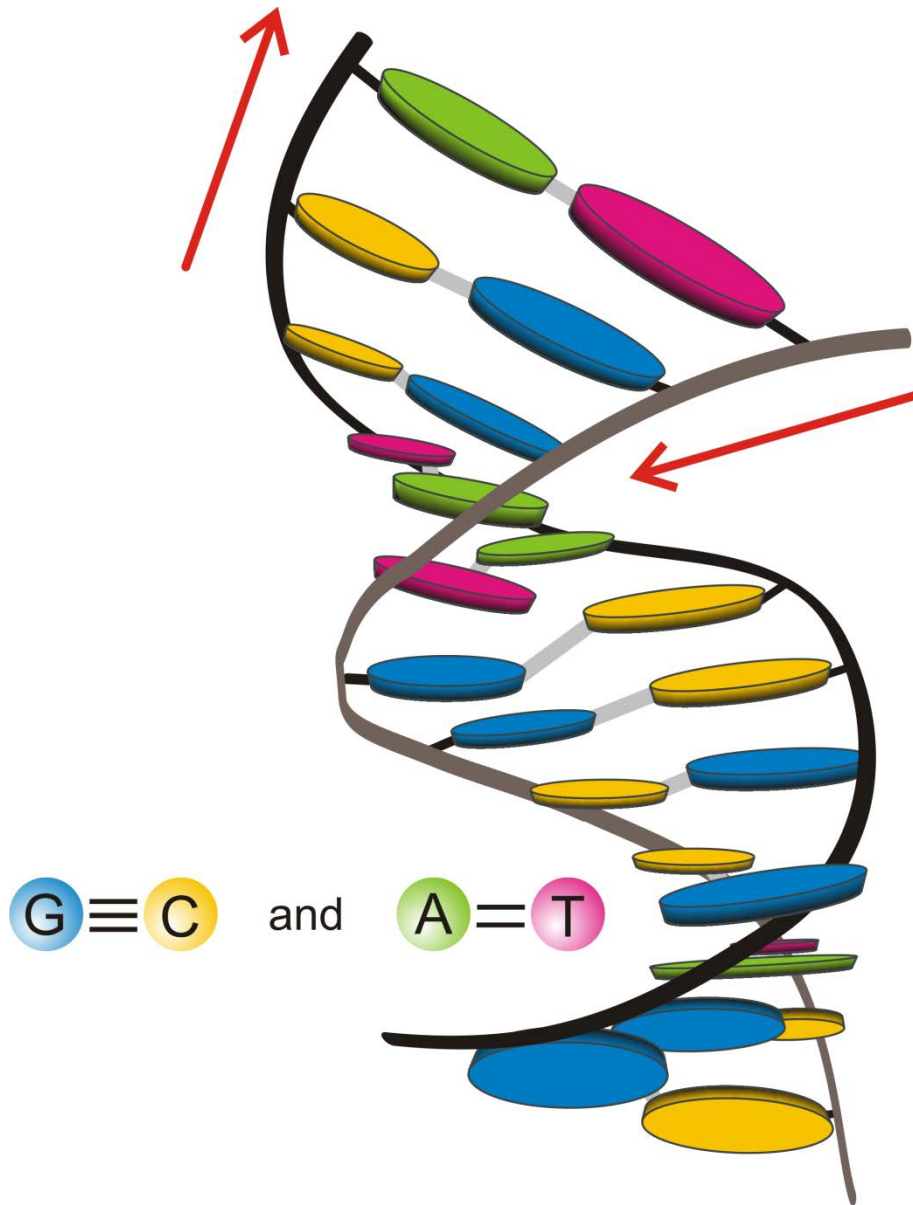
„Genetics is the first biological science which got in the position in which physics has been for many years. One can justifiably speak about such a thing as theoretical mathematical genetics, and experimental genetics, just as in physics. There are some mathematical geniuses who work out what to an ordinary person seems as a fantastic kind of theory. This fantastic kind of theory nevertheless leads to experimentally verifiable prediction, which an experimental physicist then has to test the validity of. Since the times of Wright, Haldane, and Fisher, evolutionary genetics has been in a similar position.“

Variation II: Mutation



$$f_1 = 1, f_2 = 2, f_3 = 3, f_4 = 7$$

Vor der Entwicklung der Molekularbiologie wurde Mutation als ein "Deus ex Machina" behandelt

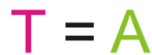
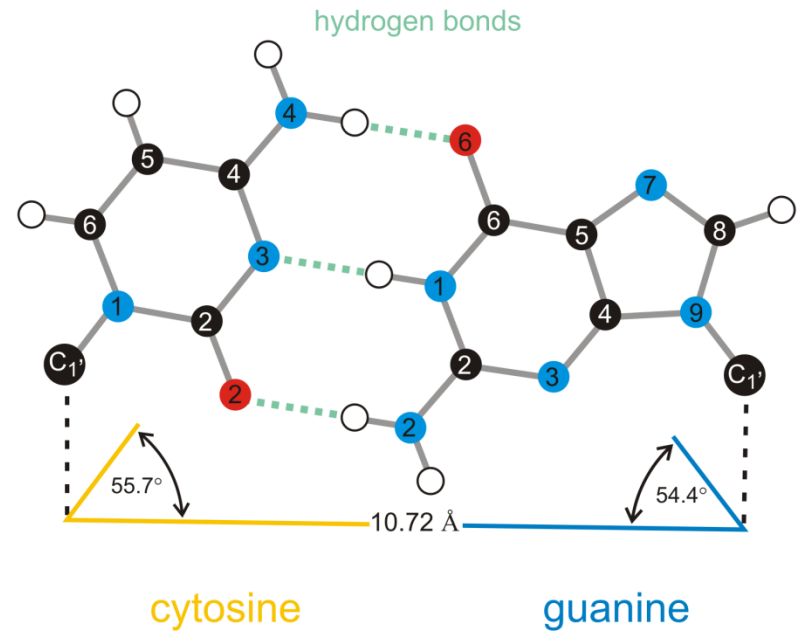


James D. Watson, 1928- , and Francis Crick, 1916-2004,
Nobel Preis 1962

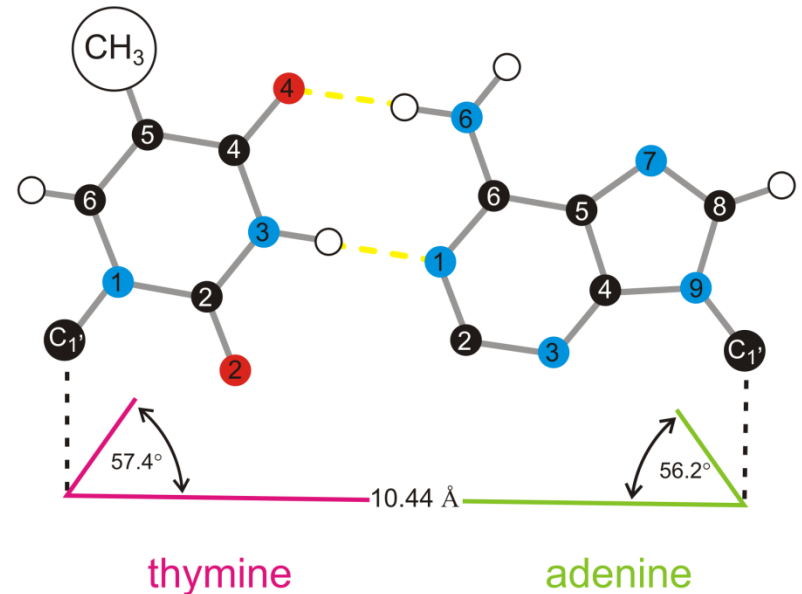
Die dreidimensionale Struktur eines
kleinen Stückes der B-DNA

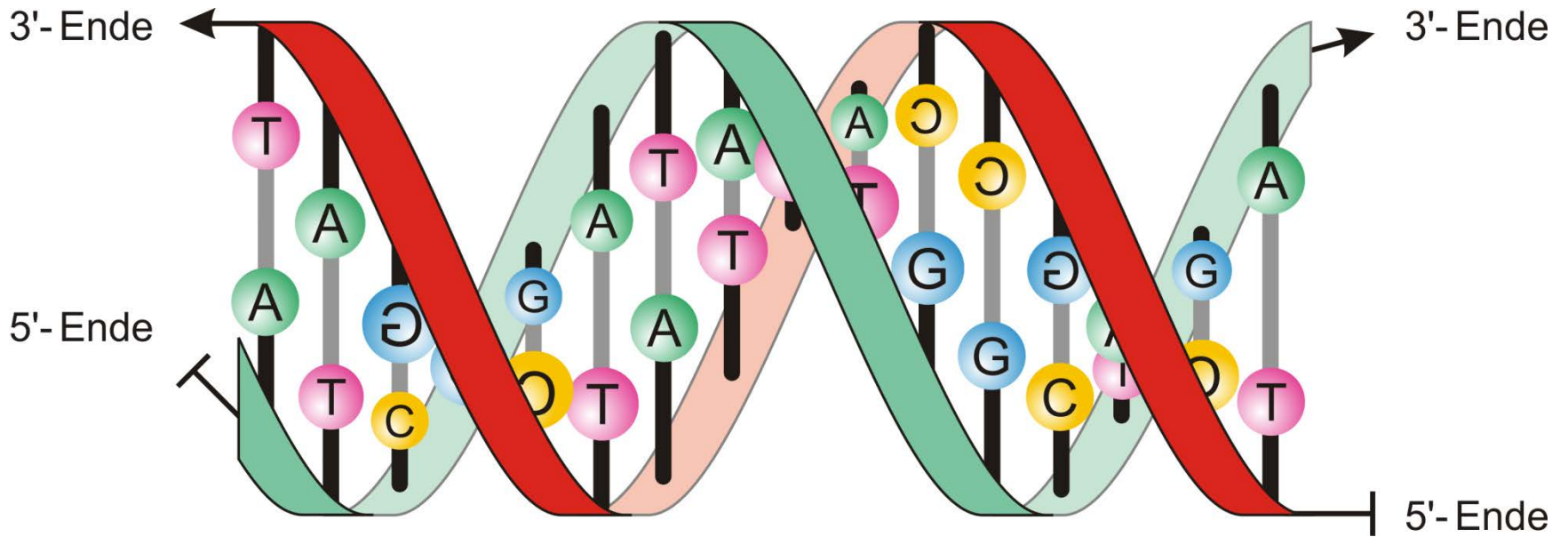


Obwohl die Wechselwirkungen mit G viel stärker sind als alle anderen Wechselwirkungen zwischen Nukleotidbasen, bilden A=T und G≡C gleichberechtigte Basenpaare.

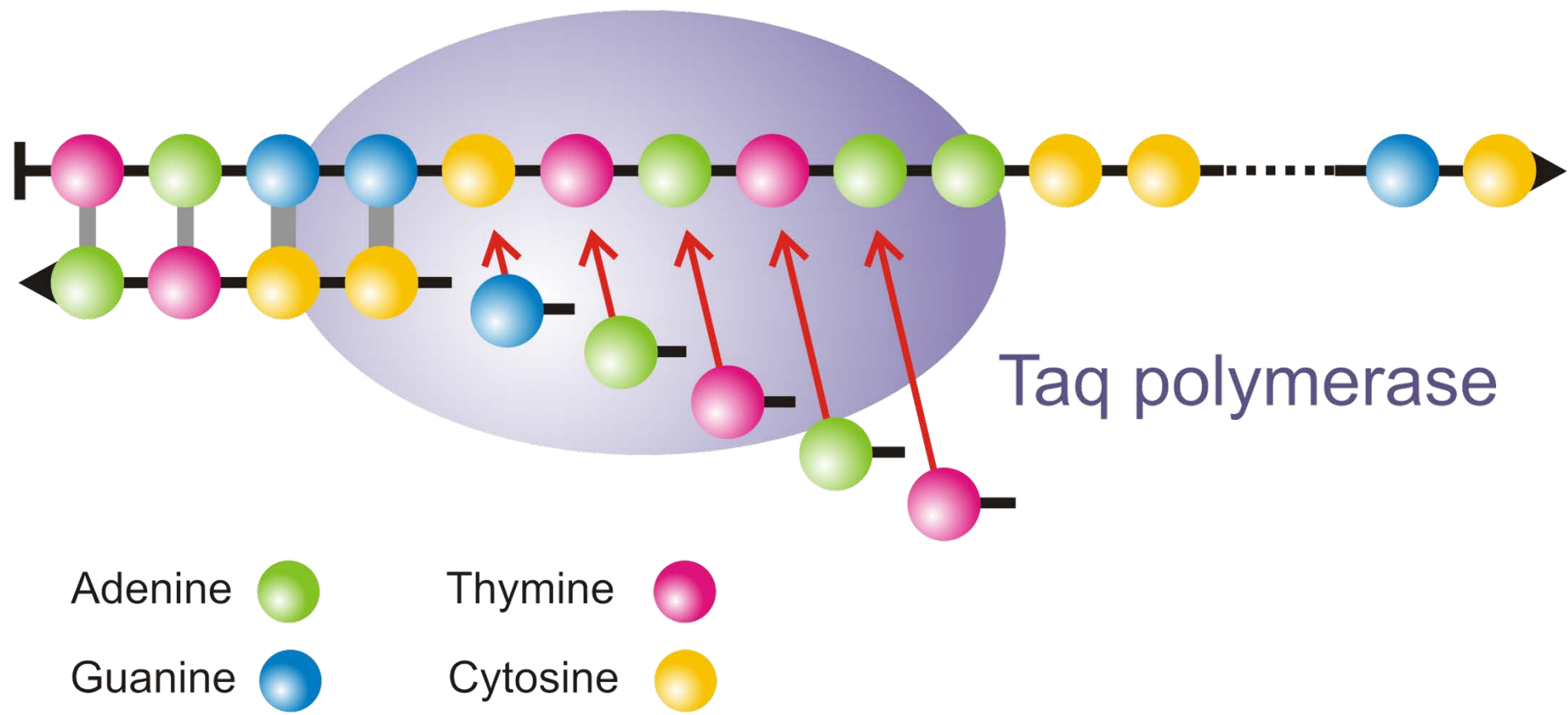


Digitalisierung der Chemie:
The unique assignment of nucleotides in base pairs.

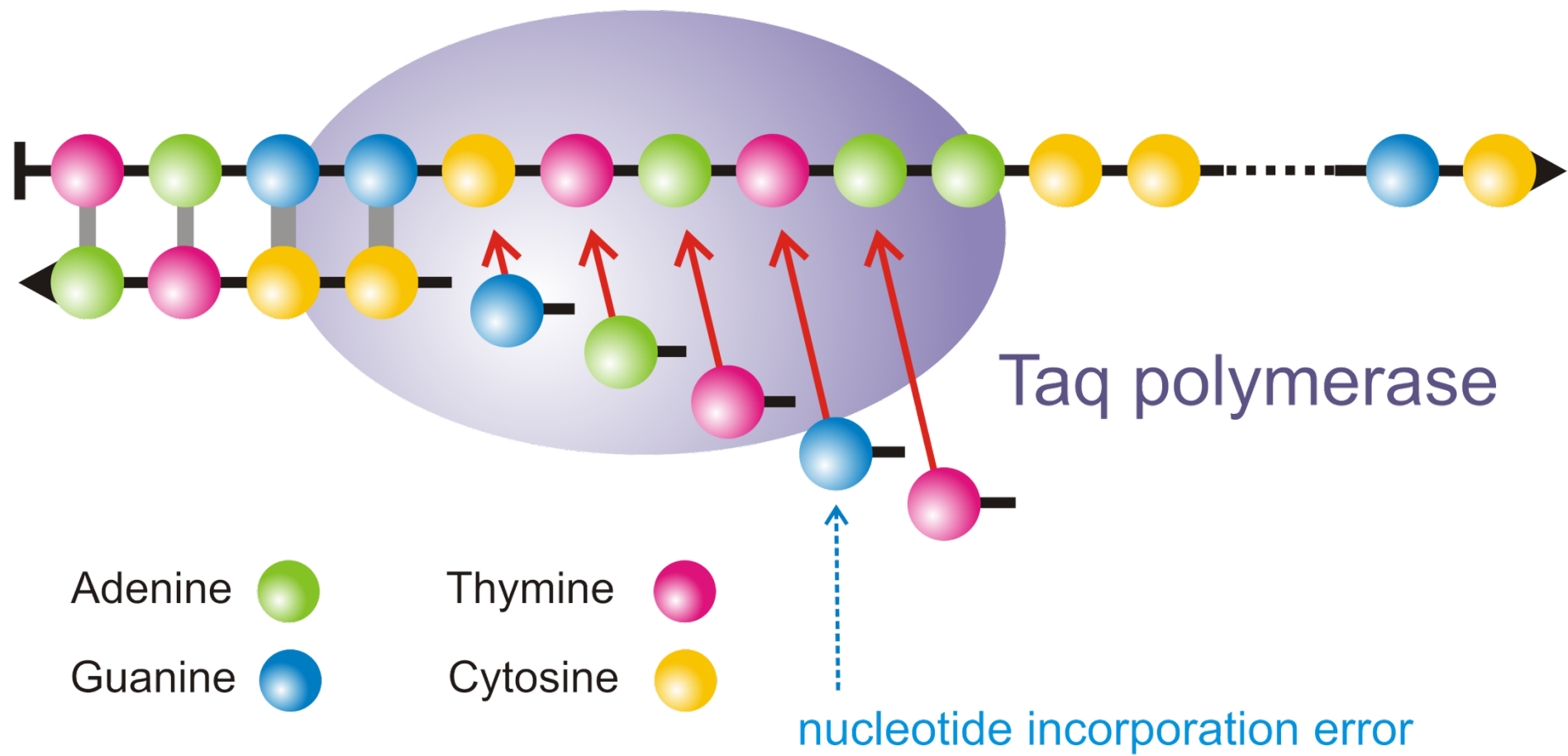




Die B-Form der DNA-Doppelhelix



The logic of DNA (or RNA) replication and mutation



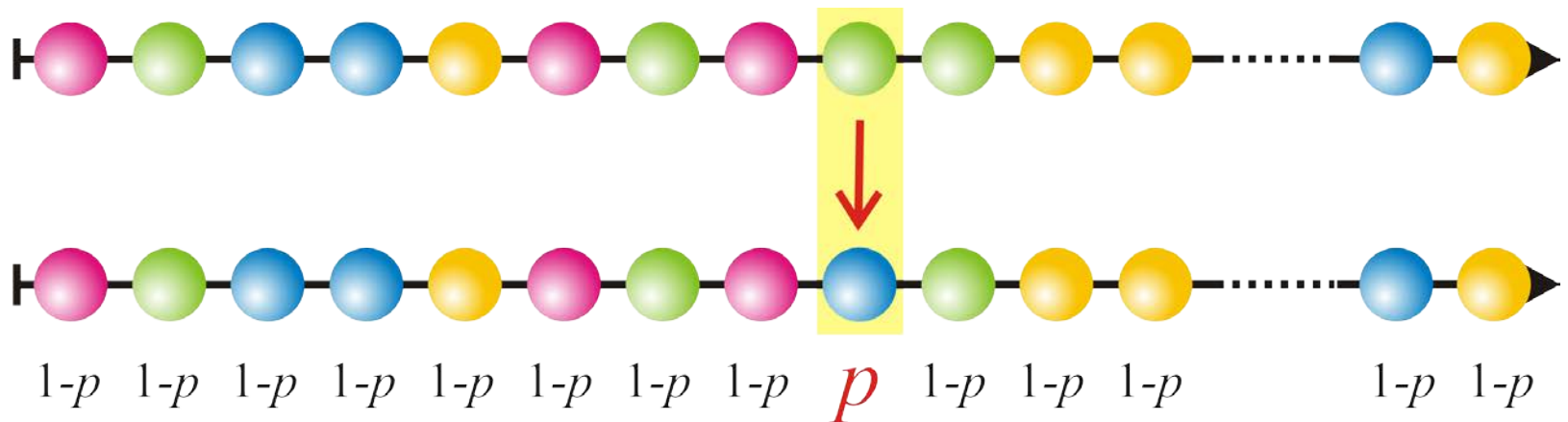
p mutation rate per site and replication

The logic of DNA (or RNA) replication and mutation

$$Q_{ij} = (1 - p)^{n - d_H(X_i, X_j)} p^{d_H(X_i, X_j)}$$

p = Fehler- oder Mutationsrate pro Replikation und Nukleotid

n = Kettenlänge und $d_H(X_i, X_j)$ = Hammingabstand



Punktmutation

Von der Theorie zur Anwendung

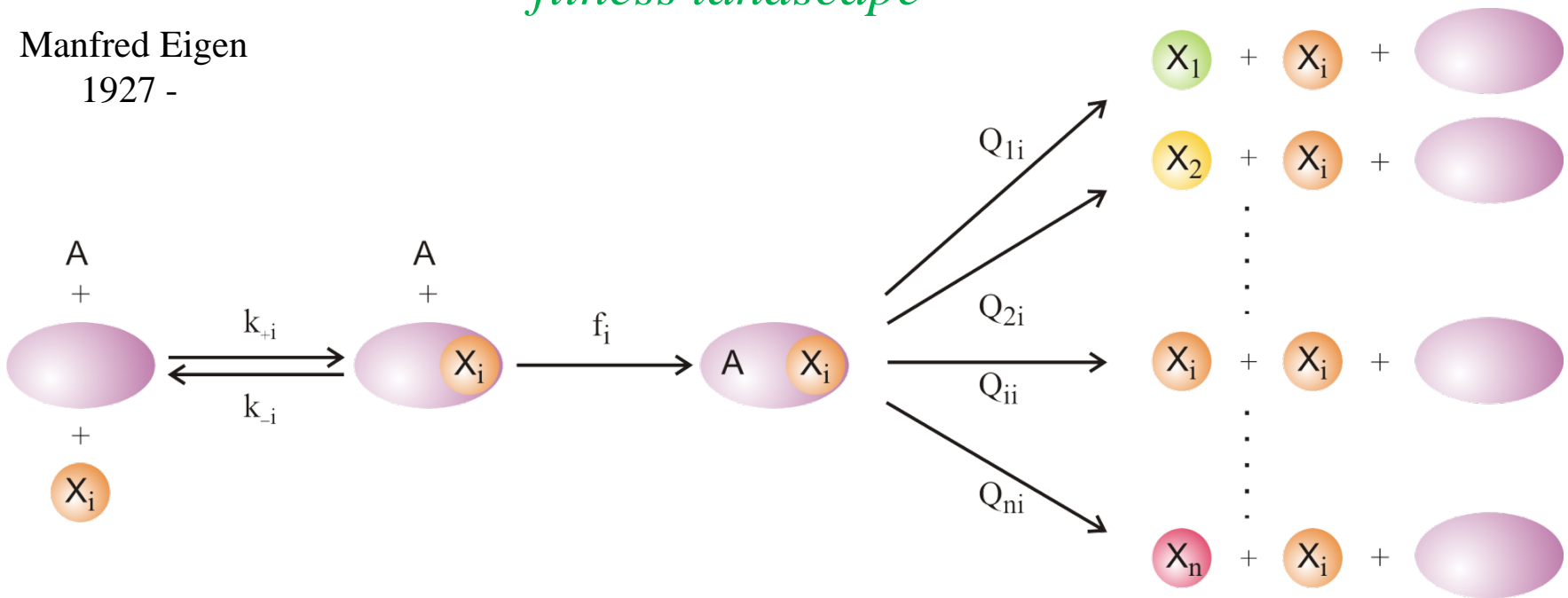


Manfred Eigen
1927 -

$$\frac{dx_j}{dt} = \sum_{i=1}^n W_{ji} x_i - x_j \Phi; \quad j=1,2,\dots,n$$

$$W_{ji} = Q_{ji} \cdot f_i, \quad \sum_{i=1}^n x_i = 1, \quad \Phi = \sum_{i=1}^n f_i x_i$$

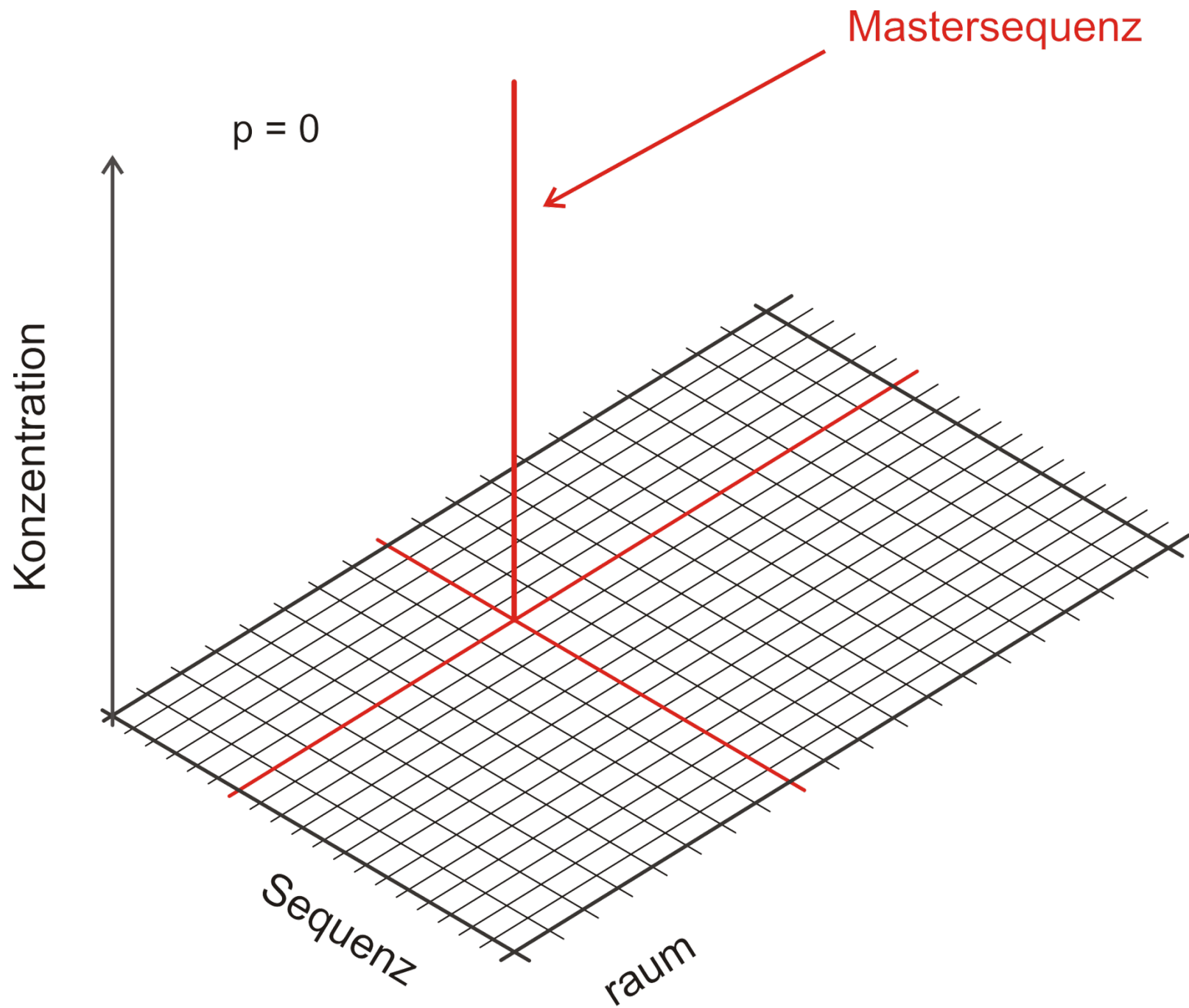
fitness landscape

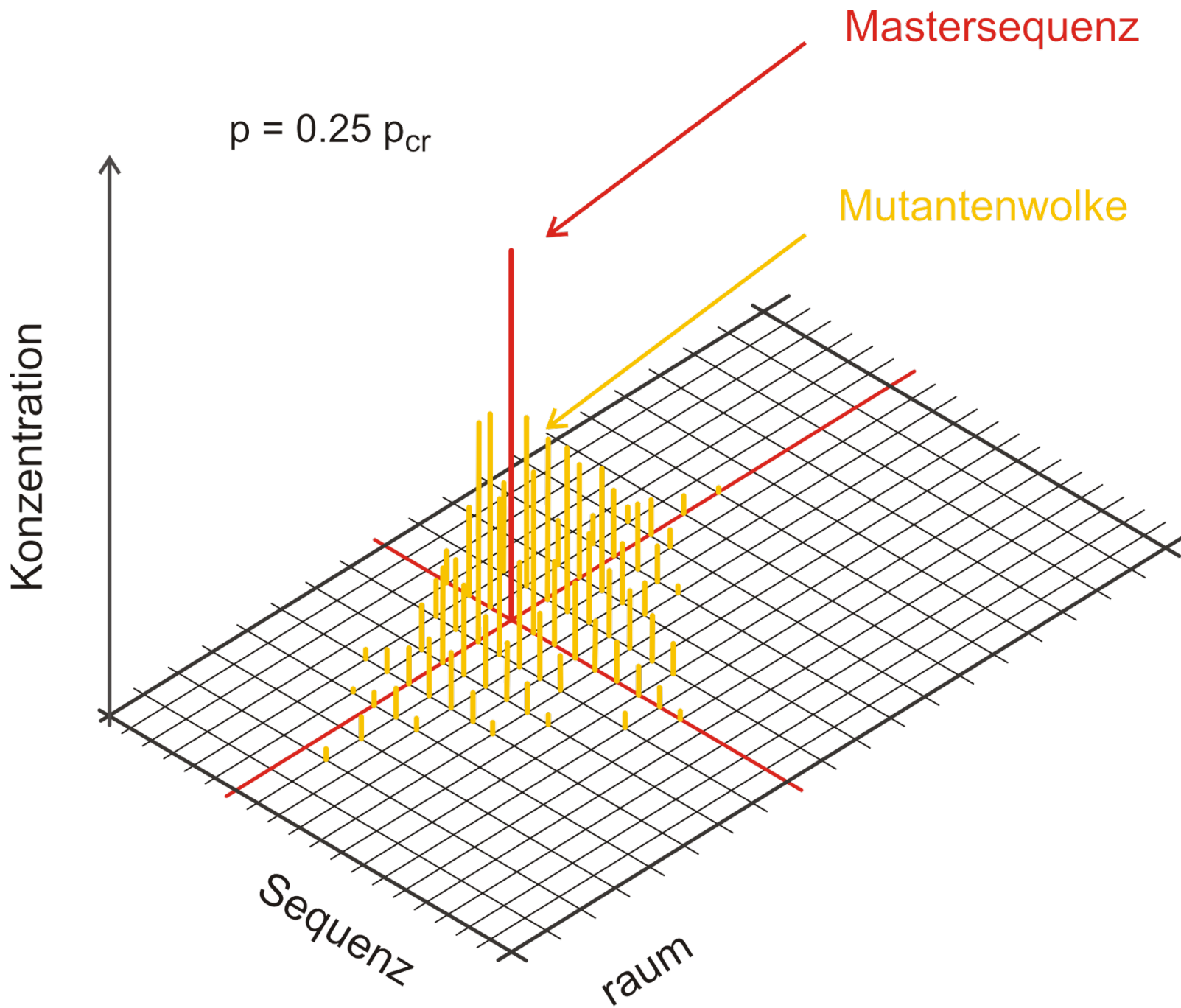


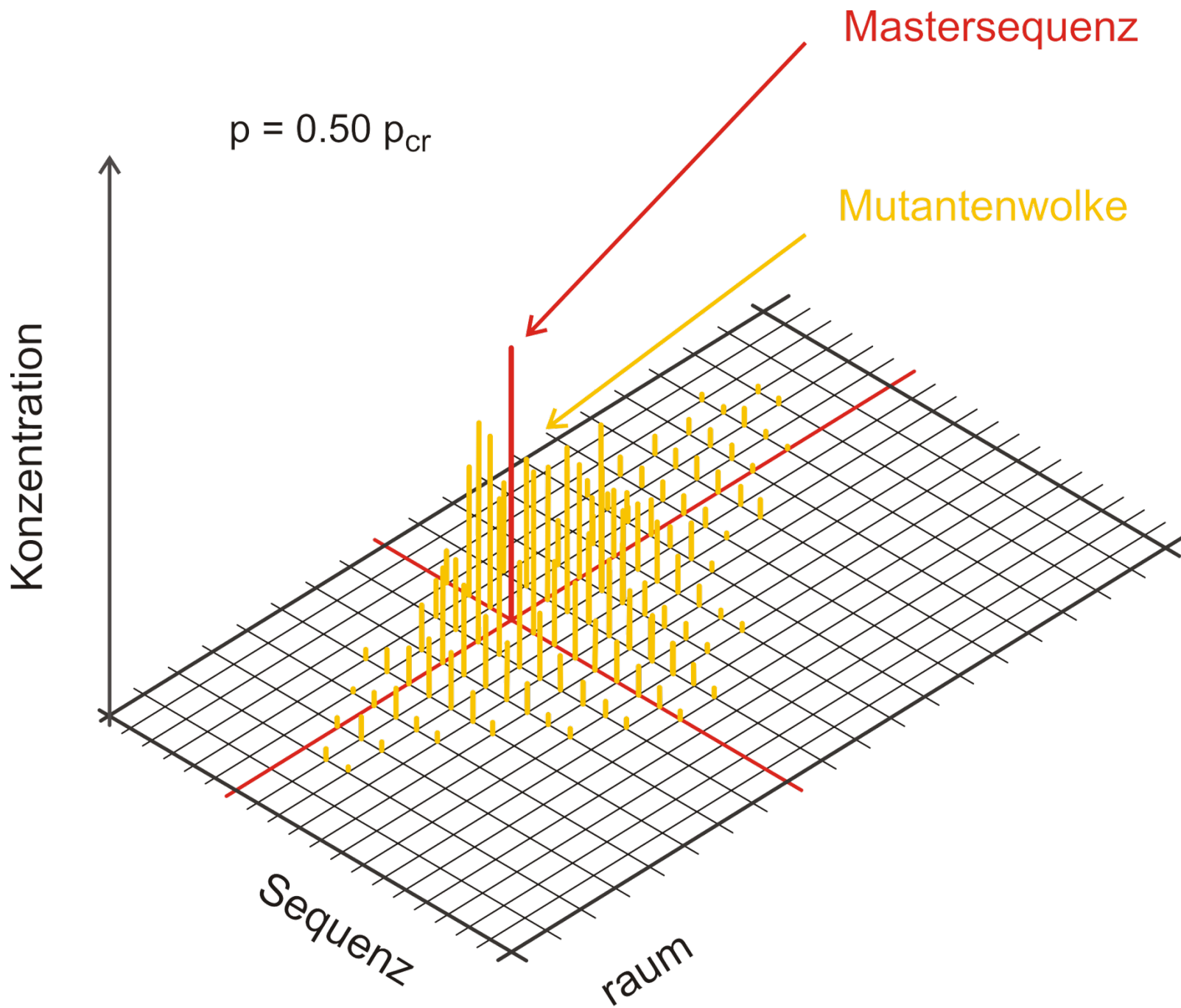
Mutation and (correct) replication as parallel chemical reactions

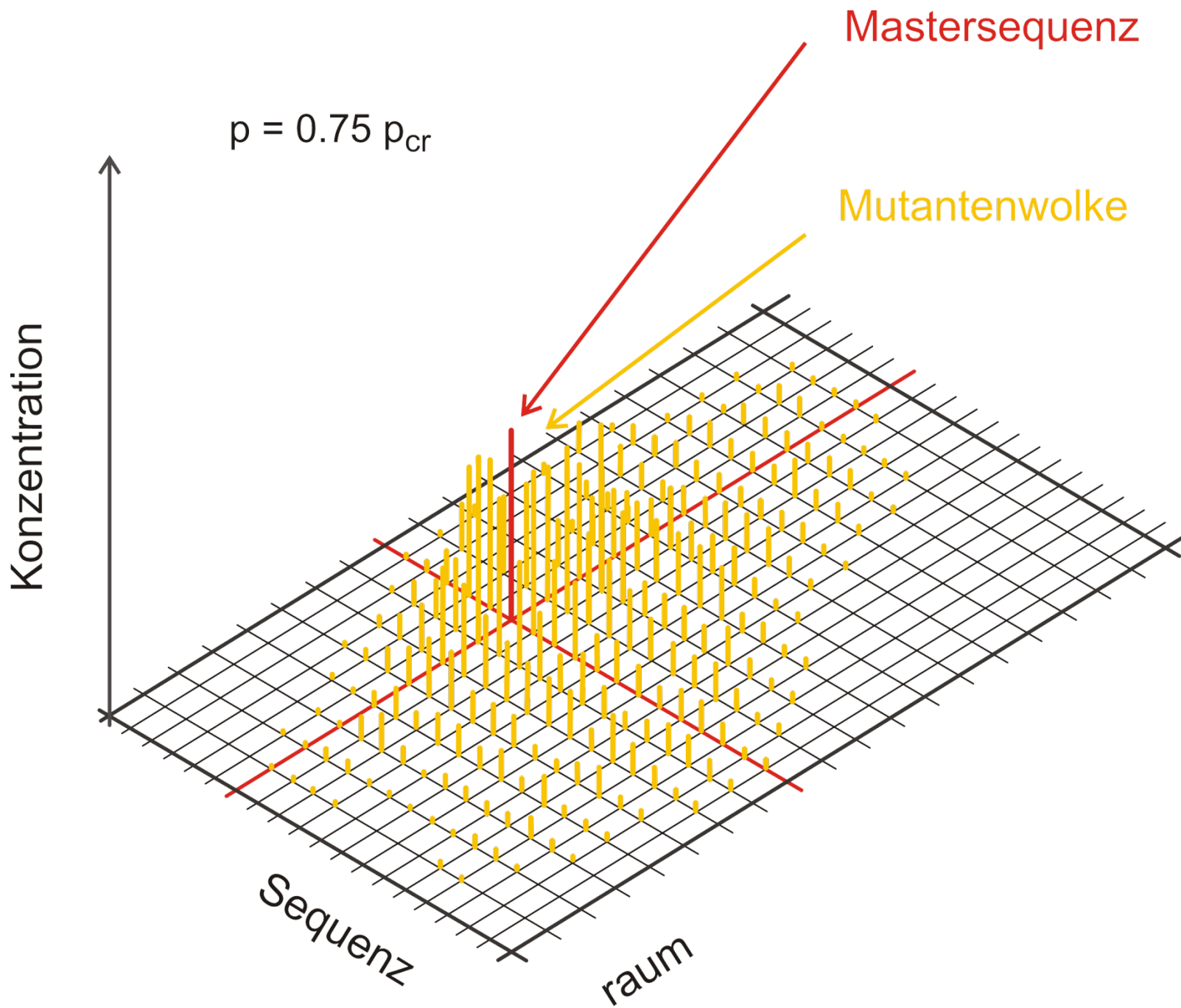
M. Eigen. 1971. *Naturwissenschaften* 58:465,

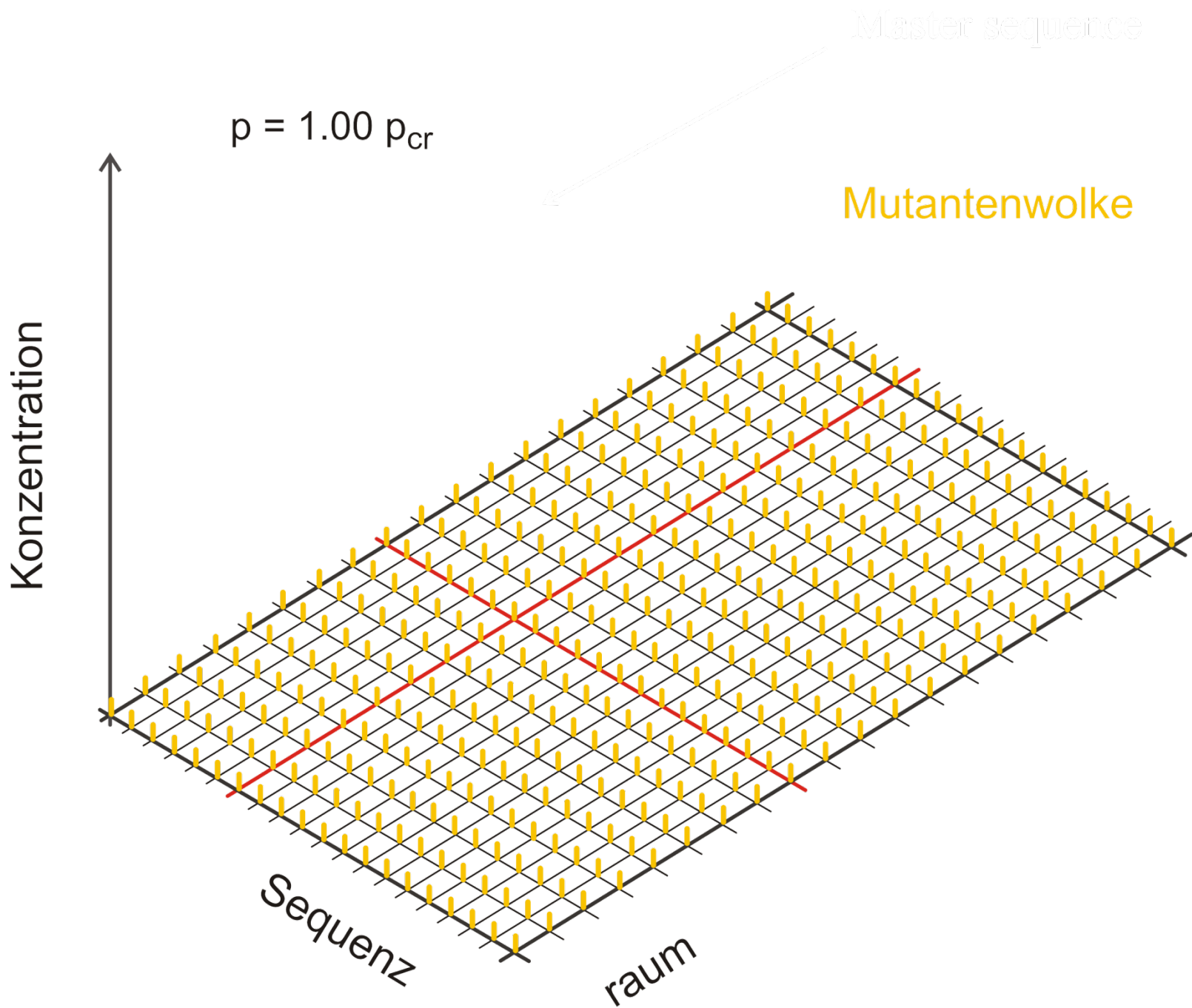
M. Eigen & P. Schuster. 1977. *Naturwissenschaften* 64:541, 65:7 und 65:341

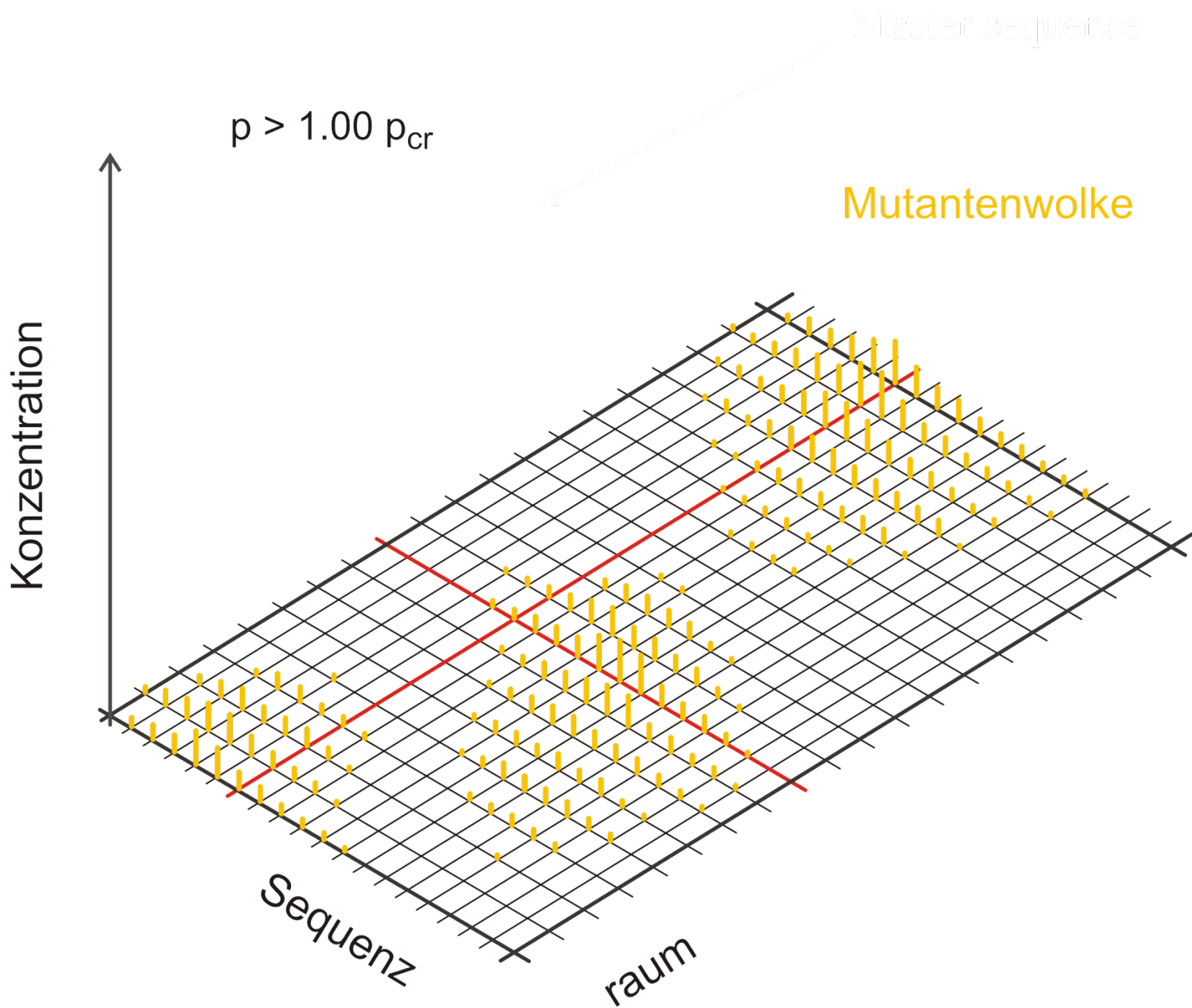


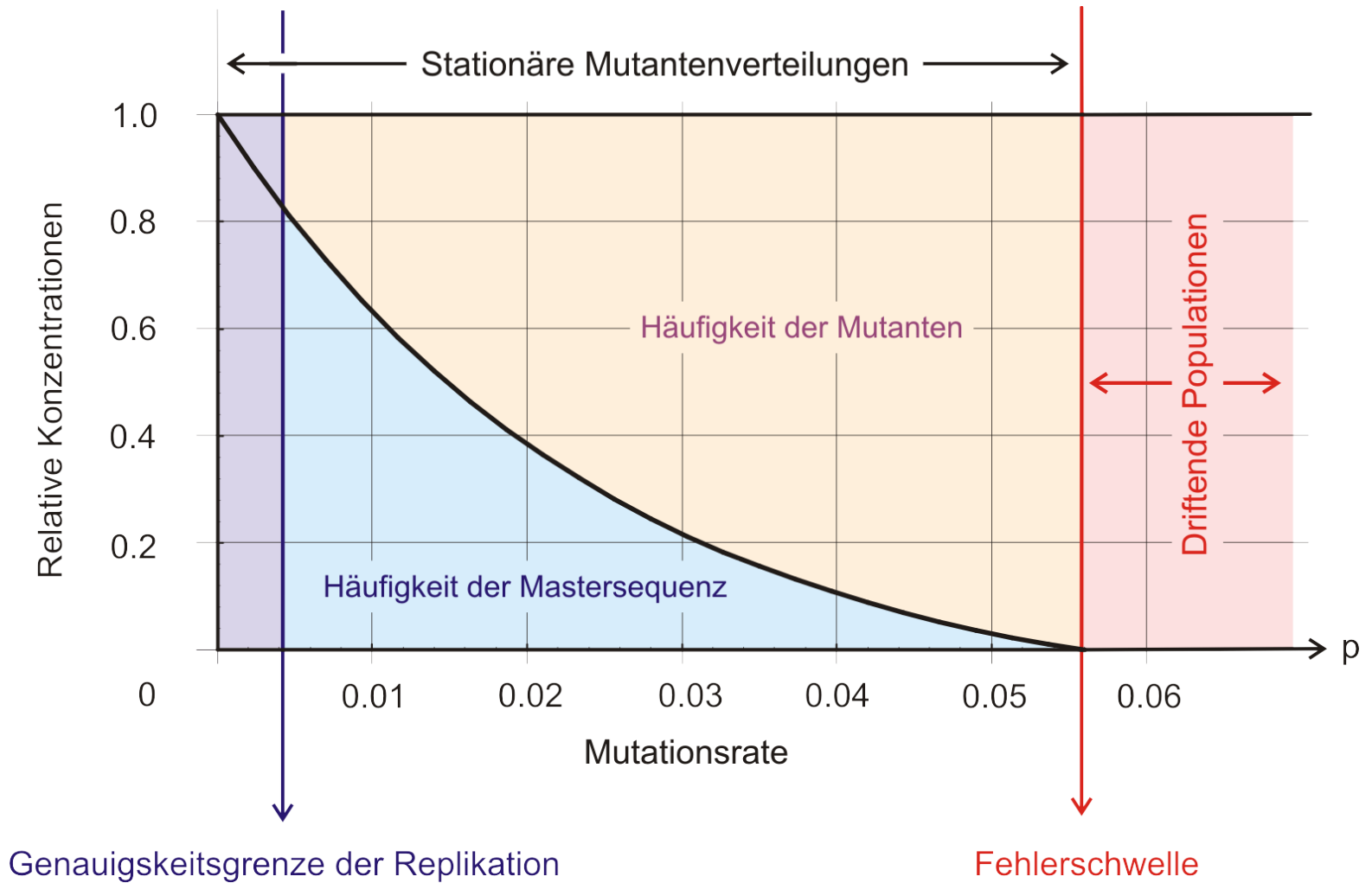




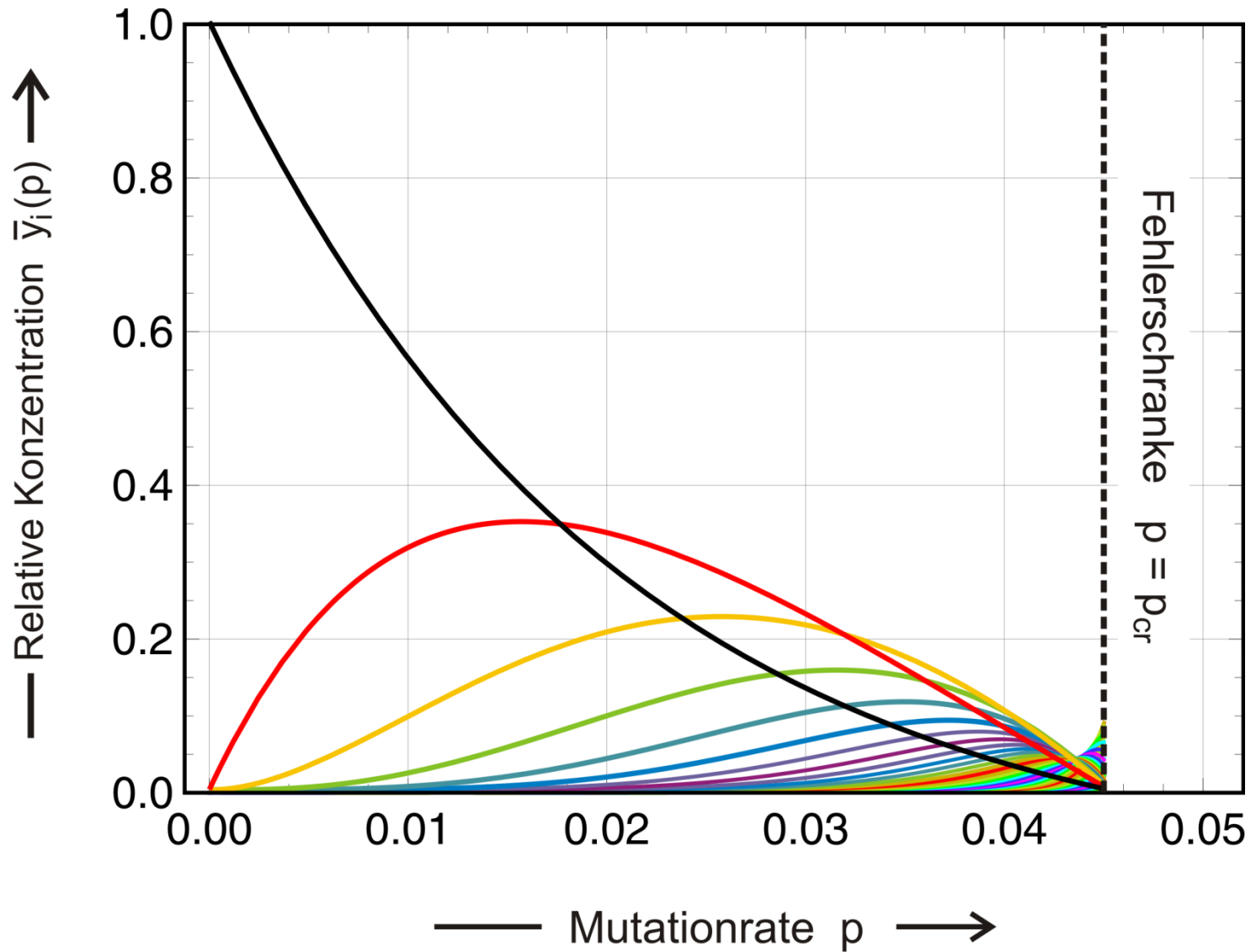




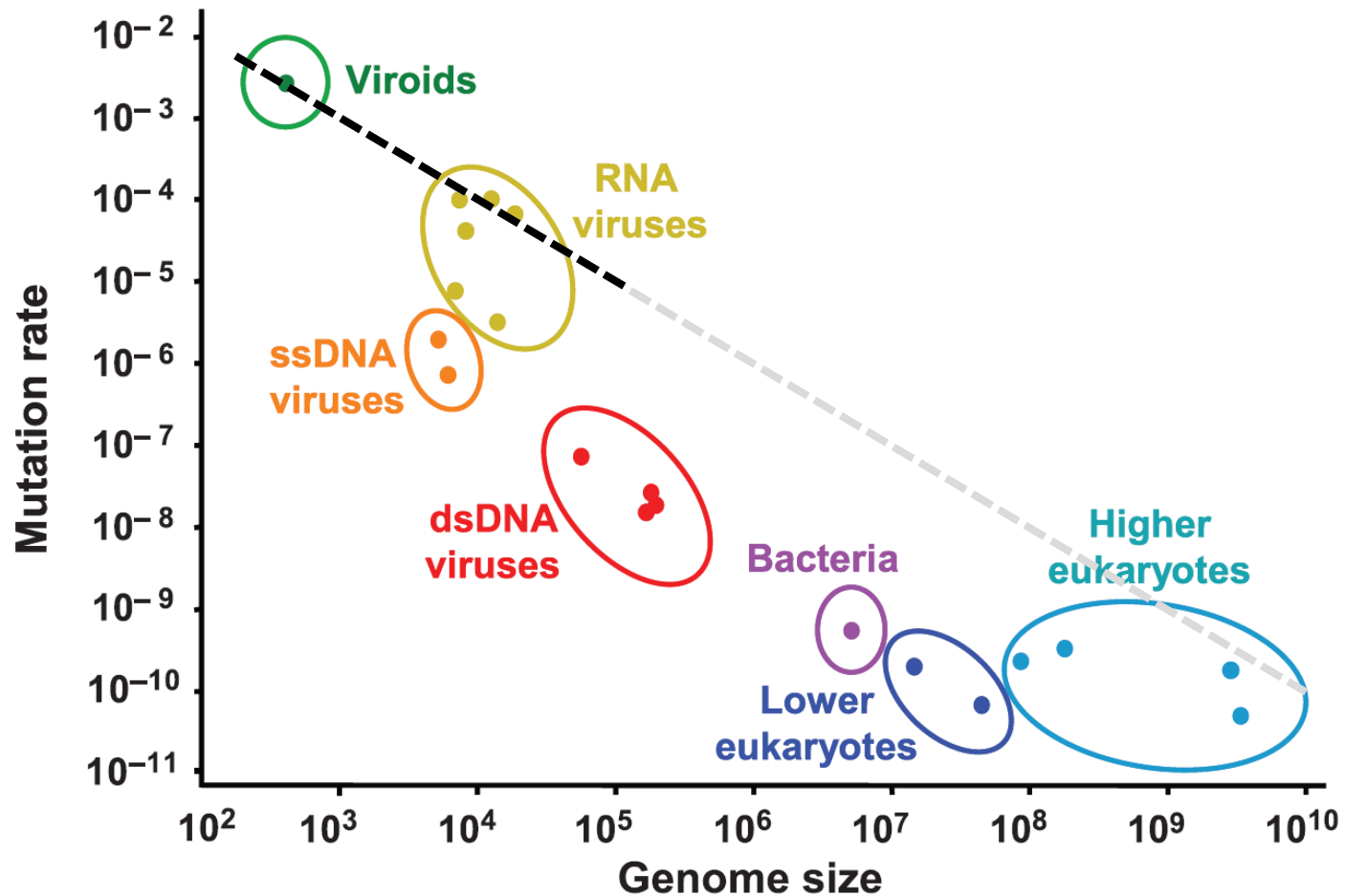




Die Fehlerschwelle bei Replikation und Mutation



Die stationäre Mutantverteilung als Funktion der Mutationsrate p



Selma Gago, Santiago F. Elena, Ricardo Flores, Rafael Sanjuán. 2009, Extremely high mutation rate of a hammerhead viroid. *Science* 323:1308.

Mutation rate and genome size



Three necessary conditions for Darwinian evolution are:

1. **Multiplication,**
2. **Variation,** and
3. **Selection.**

Charles Darwin, 1809-1882

All three conditions are fulfilled not only by cellular organisms but also by **nucleic acid molecules** - DNA or RNA - **in** suitable **cell-free experimental assays**:

Darwinian evolution in the test tube

Molecular Evolution

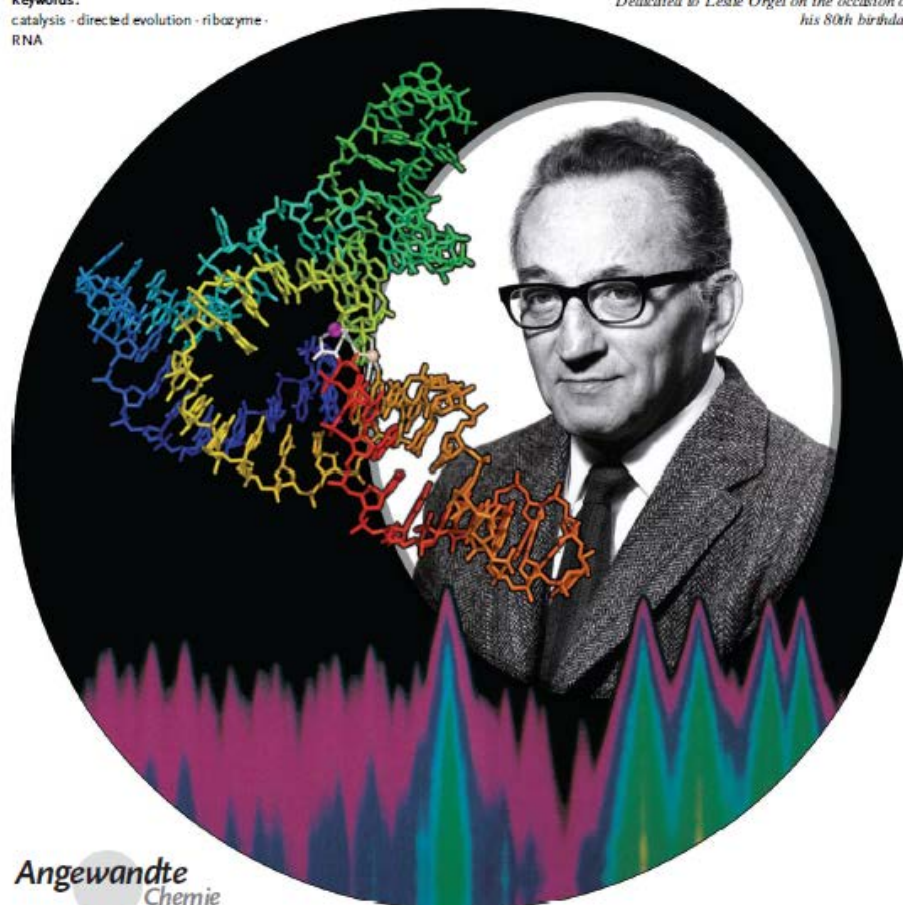
Forty Years of In Vitro Evolution**

Gerald F. Joyce*

Keywords:

catalysis · directed evolution · ribozyme · RNA

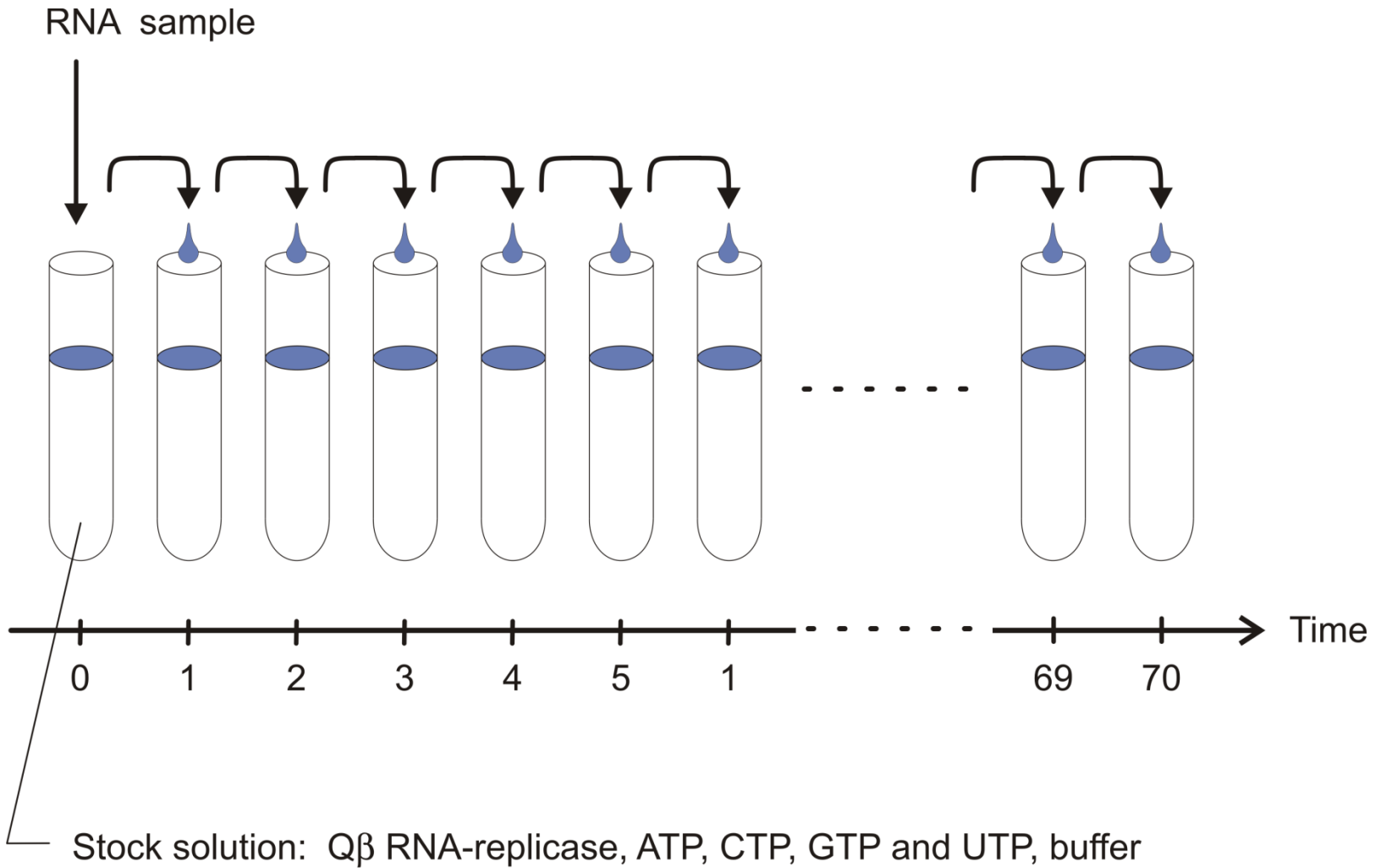
Dedicated to Leslie Orgel on the occasion of his 80th birthday



Sol Spiegelman,
1914 - 1983

Evolution im Reagenzglas:

G.F. Joyce, *Angew. Chem. Int. Ed.*
46 (2007), 6420-6436



Anwendung der Technik des seriellen Transfers zur Evolution von RNA im Reagenzglas

Reproduction of the original figure of the serial transfer experiment with Q β RNA

D.R.Mills, R.L.Peterson, S.Spiegelman,
*An extracellular Darwinian experiment
 with a self-duplicating nucleic acid
 molecule.* Proc.Natl.Acad.Sci.USA
58 (1967), 217-224

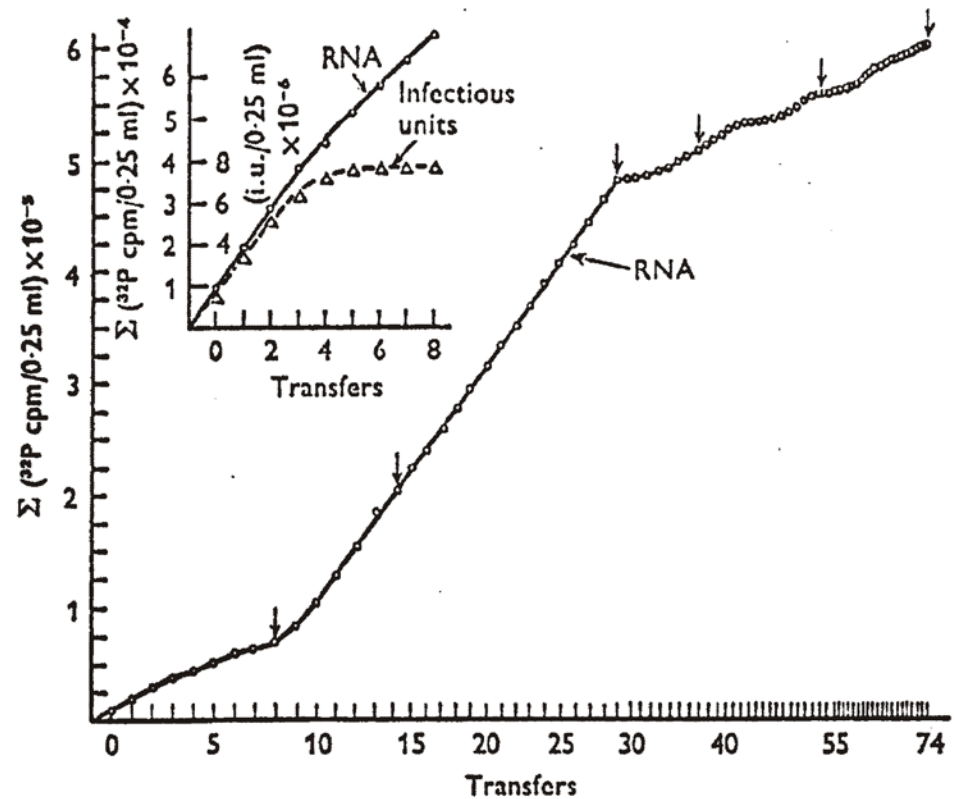
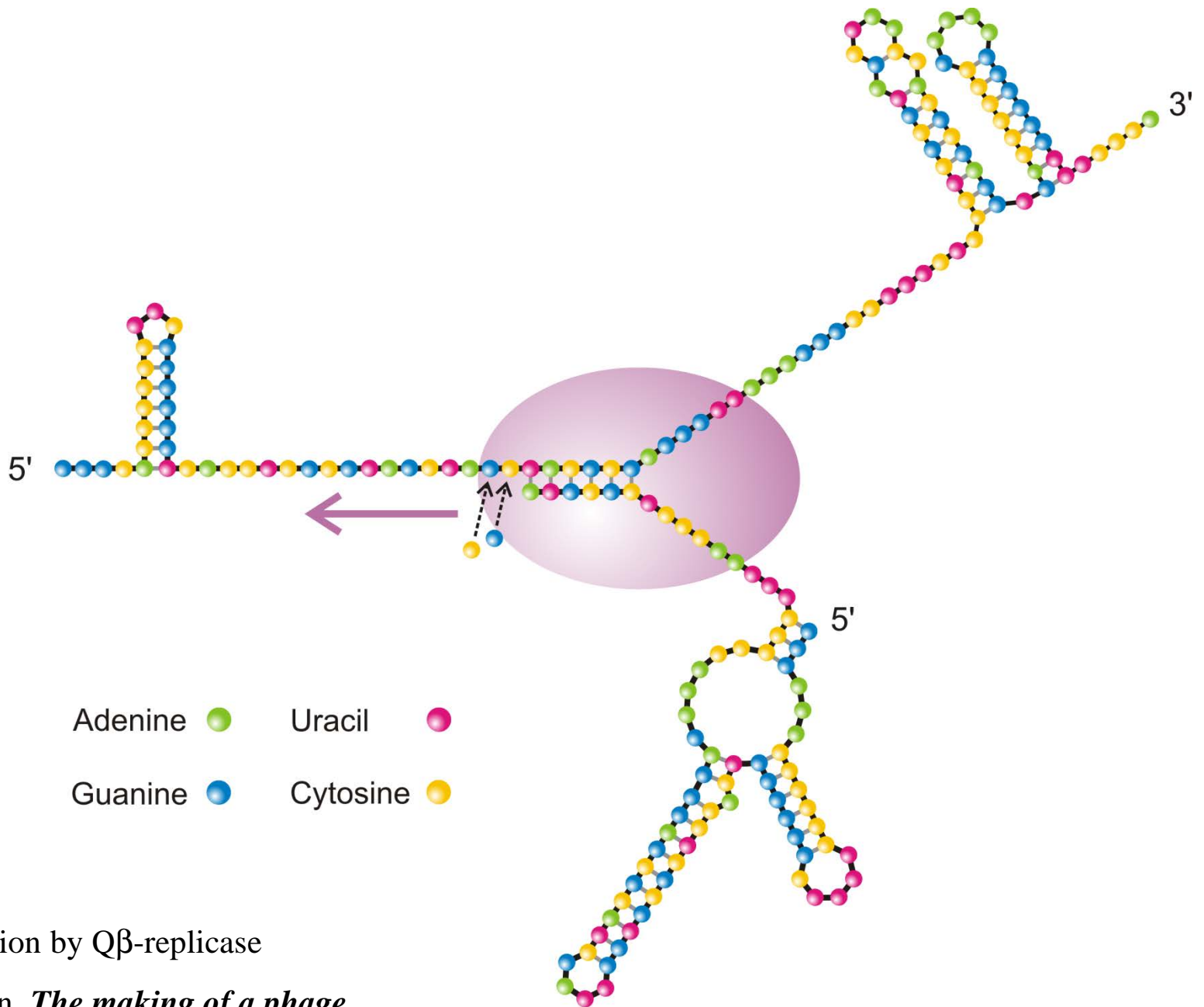


Fig. 9. Serial transfer experiment. Each 0.25 ml standard reaction mixture contained 40 μg of Q β replicase and ^{32}P -UTP. The first reaction (0 transfer) was initiated by the addition of 0.2 μg ts-1 (temperature-sensitive RNA) and incubated at 35 $^{\circ}\text{C}$ for 20 min, whereupon 0.02 ml was drawn for counting and 0.02 ml was used to prime the second reaction (first transfer), and so on. After the first 13 reactions, the incubation periods were reduced to 15 min (transfers 14-29). Transfers 30-38 were incubated for 10 min. Transfers 39-52 were incubated for 7 min, and transfers 53-74 were incubated for 5 min. The arrows above certain transfers (0, 8, 14, 29, 37, 53, and 73) indicate where 0.001-0.1 ml of product was removed and used to prime reactions for sedimentation analysis on sucrose. The inset examines both infectious and total RNA. The results show that biologically competent RNA ceases to appear after the 4th transfer (Mills *et al.* 1967).



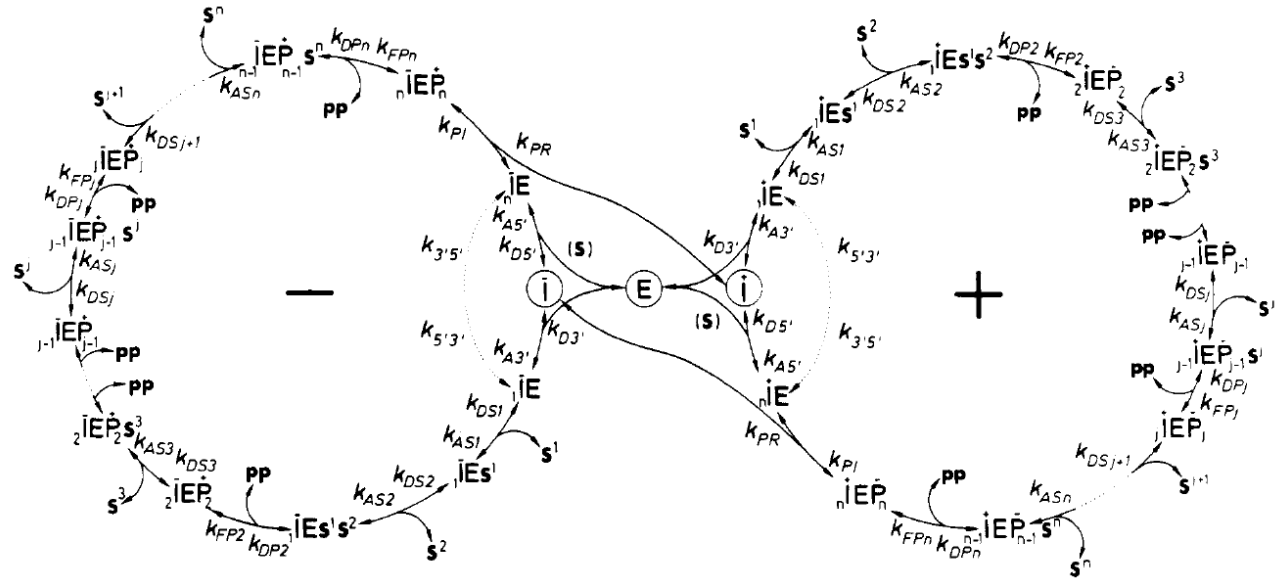
RNA replication by Q β -replicase

C. Weissmann, *The making of a phage.*

FEBS Letters **40** (1974), S10-S18

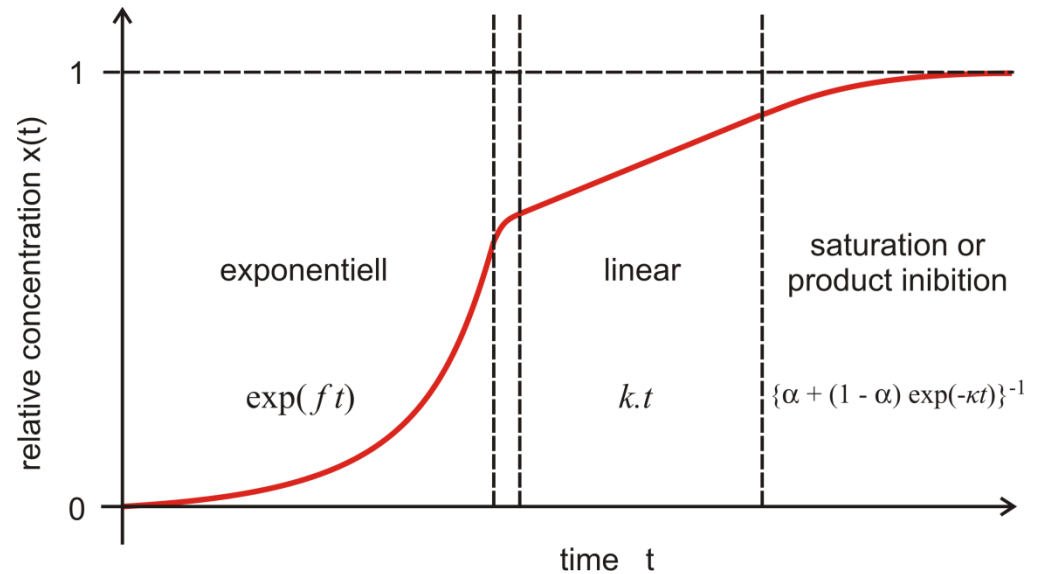


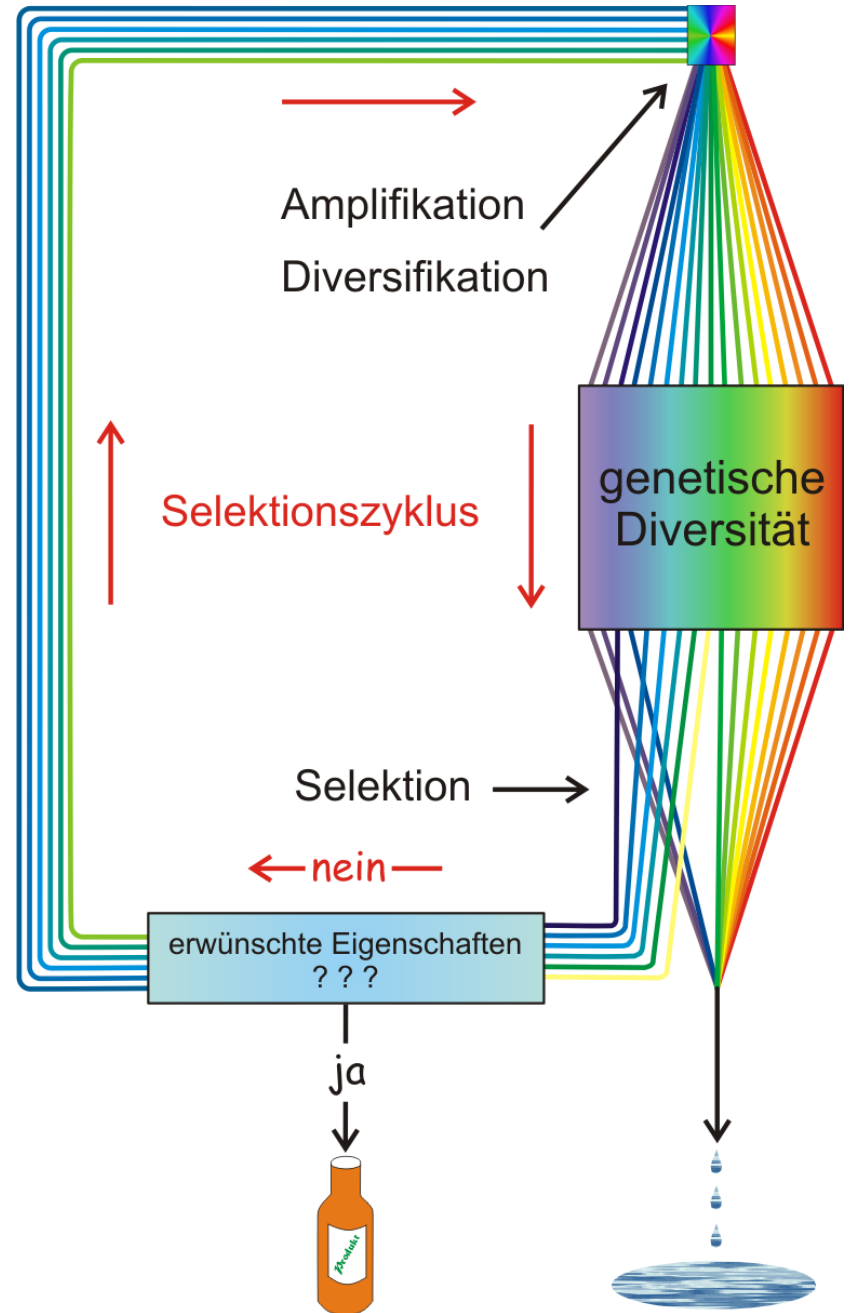
Christof K. Biebricher,
1941-2009



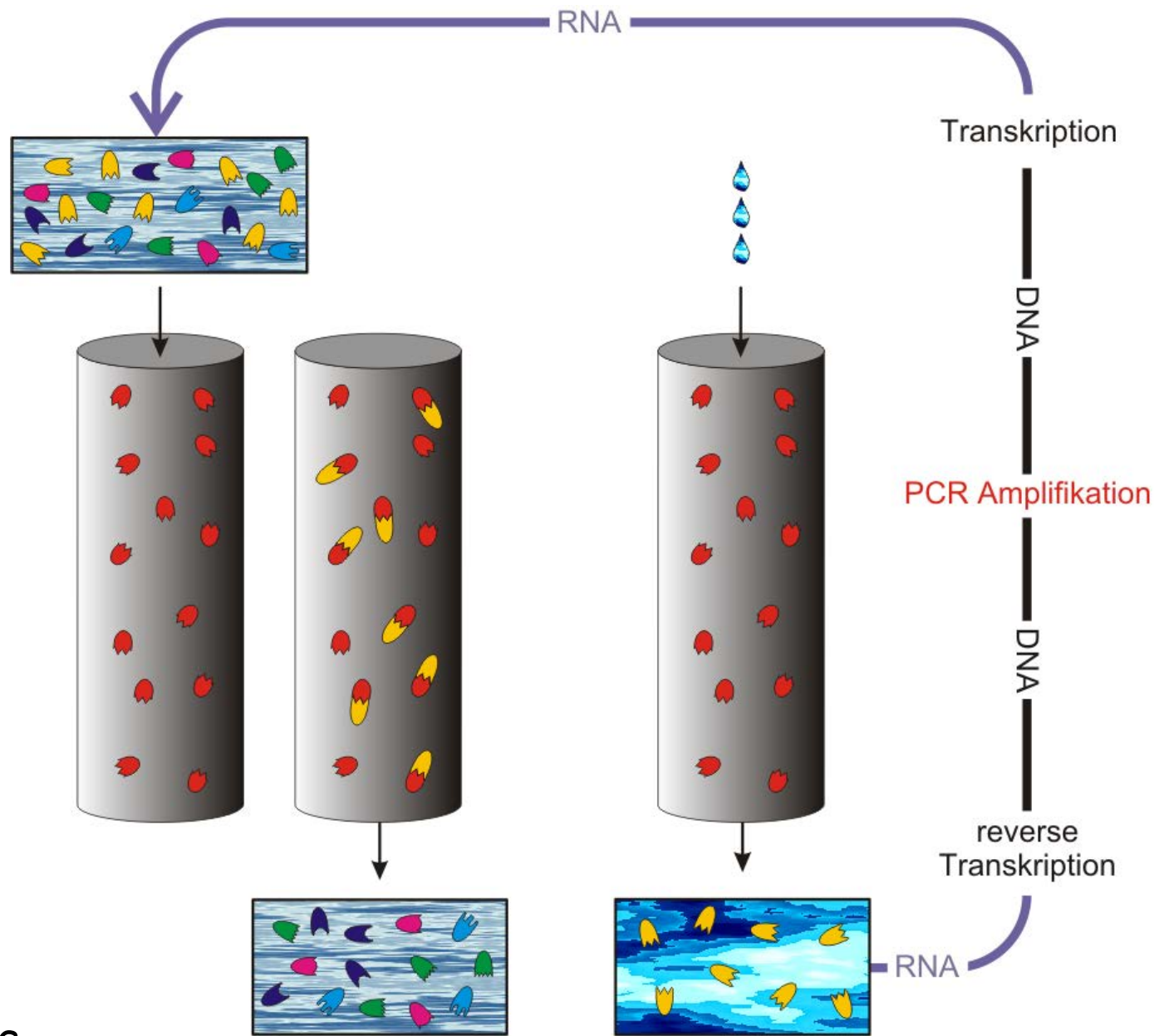
Kinetik der RNA Replikation

C.K. Biebricher, M. Eigen, W.C. Gardiner, Jr.
Biochemistry **22**:2544-2559, 1983

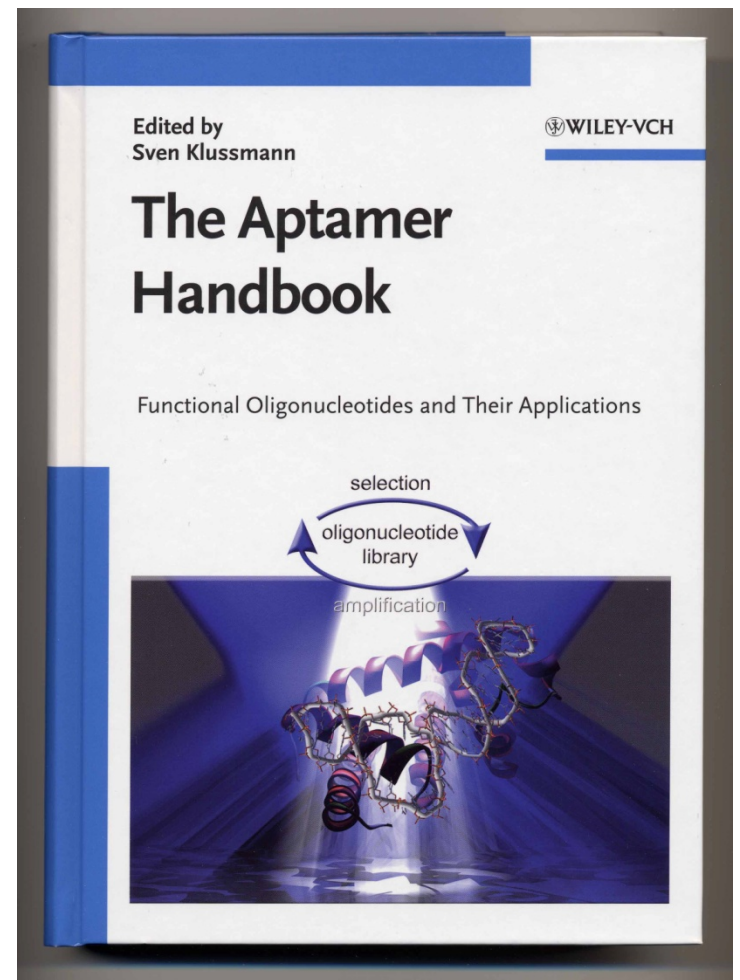
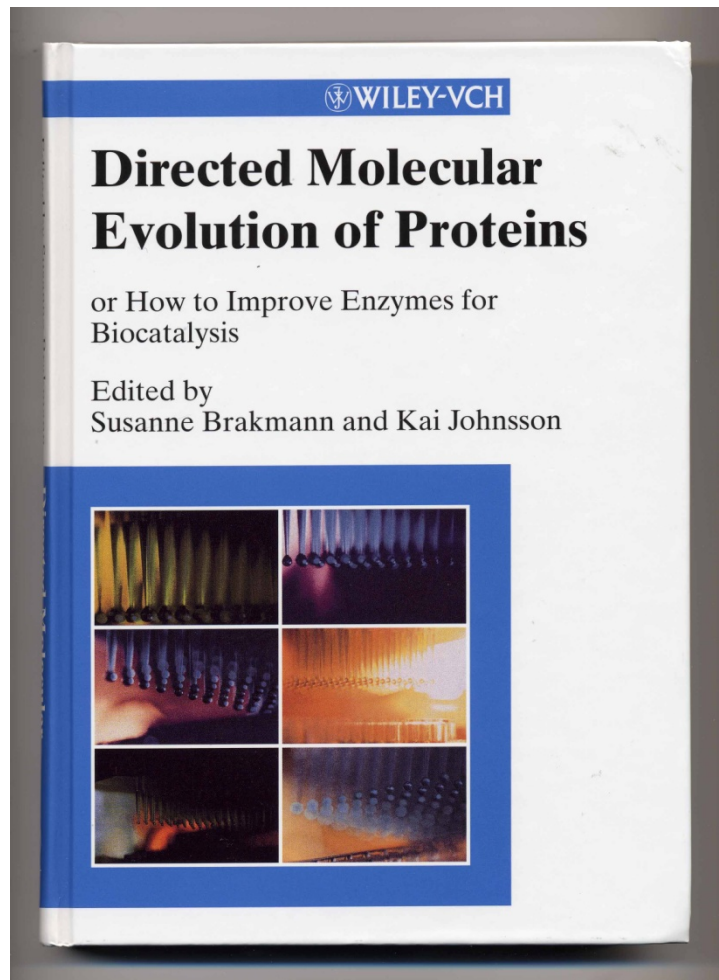




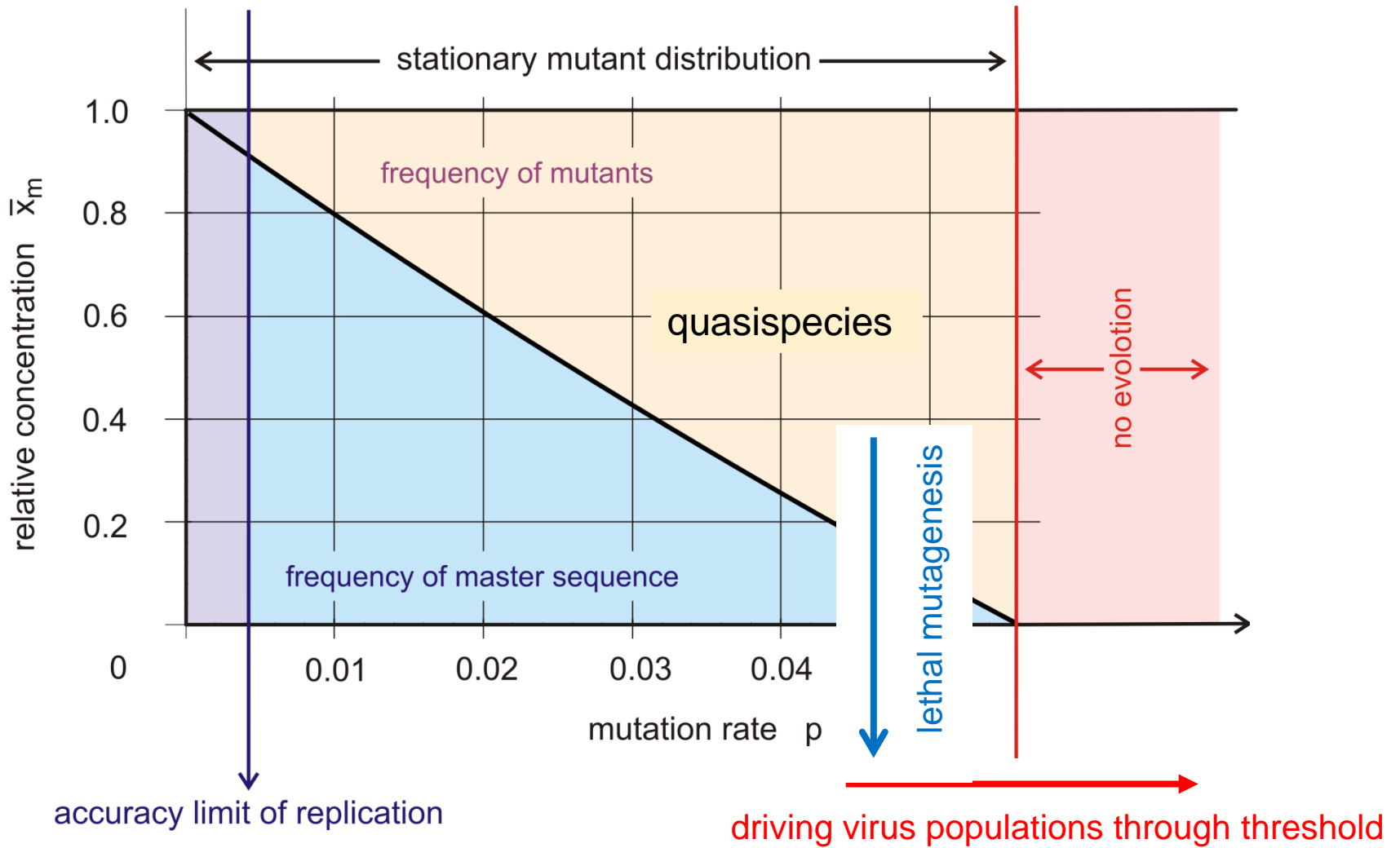
Das Prinzip der evolutionären Biotechnologie



The PCR technique as a selection tool



Application of molecular evolution to problems in biotechnology



The error threshold in replication

Preface

Antiviral strategy on the horizon

Error catastrophe had its conceptual origins in the middle of the XXth century, when the consequences of mutations on enzymes involved in protein synthesis, as a theory of aging. In those times biological processes were generally perceived differently from today. Infectious diseases were regarded as a fleeting nuisance which would be eliminated through the use of antibiotics and antiviral agents. Microbial variation, although known in some cases, was not thought to be a significant problem for disease control. Variation in differentiated organisms was seen as resulting essentially from exchanges of genetic material associated with sexual reproduction. The problem was to unveil the mechanisms of inheritance, expression of genetic information and metabolism. Few saw that genetic change is occurring at present in all organisms, and still fewer recognized Darwinian principles as essential to the biology of pathogenic viruses and cells. Population geneticists rarely used bacteria or viruses as experimental systems to define concepts in biological evolution. The extent of genetic polymorphism among individuals of the same biological species came as a surprise when the first results on comparison of electrophoretic mobility of enzymes were obtained. With the advent of *in vitro* DNA recombination, and rapid nucleic acid sequencing techniques, molecular analyses of genomes reinforced the conclusion of extreme inter-individual genetic variation within the same species. Now, due largely to spectacular progress in comparative genomics, we see cellular DNAs, both prokaryotic and eukaryotic, as highly dynamic. Most cellular processes, including such essential information-bearing and transferring events as genome replication, transcription and translation, are increasingly perceived as inherently inaccurate. Viruses, and in particular RNA viruses, are among the most extreme examples of exploitation of replication inaccuracy for survival.

Error catastrophe, or the loss of meaningful genetic information through excess genetic variation, was formulated in quantitative terms as a consequence of quasispecies theory, which was first developed to explain self-organization and adaptability of primitive replicons in early stages of life. Recently, a conceptual extension of error catastrophe that could be defined as “induced genetic deterioration” has emerged as

a possible antiviral strategy. This is the topic of the current special issue of *Virus Research*.

Few would nowadays doubt that one of the major obstacles for the control of viral disease is short-term adaptability of viral pathogens. Adaptability of viruses follows the same Darwinian principles that have shaped biological evolution over eons, that is, repeated rounds of reproduction with genetic variation, competition and selection, often perturbed by random events such as statistical fluctuations in population size. However, with viruses the consequences of the operation of these very same Darwinian principles are felt within very short times. Short-term evolution (within hours and days) can be also observed with some cellular pathogens, with subsets of normal cells, and cancer cells. The nature of RNA viral pathogens begs for alternative antiviral strategies, and forcing the virus to cross the critical error threshold for maintenance of genetic information is one of them.

The contributions to this volume have been chosen to reflect different lines of evidence (both theoretical and experimental) on which antiviral designs based on genetic deterioration inflicted upon viruses are being constructed. Theoretical studies have explored the copying fidelity conditions that must be fulfilled by any information-bearing replication system for the essential genetic information to be transmitted to progeny. Closely related to the theoretical developments have been numerous experimental studies on quasispecies dynamics and their multiple biological manifestations. The latter can be summarized by saying that RNA viruses, by virtue of existing as mutant spectra rather than defined genetic entities, remarkably expand their potential to overcome selective pressures intended to limit their replication. Indeed, the use of antiviral inhibitors in clinical practice and the design of vaccines for a number of major RNA virus-associated diseases, are currently presided by a sense of uncertainty. Another line of growing research is the enzymology of copying fidelity by viral replicases, aimed at understanding the molecular basis of mutagenic activities. Error catastrophe as a potential new antiviral strategy received an important impulse by the observation that ribavirin (a licensed antiviral nucleoside analogue) may be exerting, in some systems, its antiviral activity through enhanced mutagenesis. This has encouraged investigations on new mutagenic base analogues, some of them used in anticancer chemotherapy. Some chapters summarize these important biochemical studies on cell entry pathways and metabolism of mutagenic agents, that may find new applications as antiviral agents.

This volume intends to be basically a progress report, an introduction to a new avenue of research, and a realistic appraisal of the many issues that remain to be investigated. In this respect, I can envisage (not without many uncertainties) at least three lines of needed research: (i) One on further understanding of quasispecies dynamics in infected individuals to learn more on how to apply combinations of virus-specific mutagens and inhibitors in an effective way, finding synergistic combinations and avoiding antagonistic ones as well as severe clinical side effects. (ii) Another on a deeper understanding of the metabolism of mutagenic agents, in particular base and nucleoside analogues. This includes identification of the transporters that carry them into cells, an understanding of their metabolic processing, intracellular stability and alterations of nucleotide pools, among other issues. (iii) Still another line of needed research is the development of new mutagenic agents specific for viruses, showing no (or limited) toxicity for cells. Some advances may come from links with anticancer research, but others should result from the designs of new molecules, based on the structures of viral polymerases. I really hope that the reader finds this issue not only to be an interesting and useful review of the current situation in the field, but also a stimulating exposure to the major problems to be faced.

The idea to prepare this special issue came as a kind invitation of Ulrich Desselberger, former Editor of *Virus Research*, and then taken enthusiastically by Luis Enjuanes, recently appointed as Editor of *Virus Research*. I take this opportunity to thank Ulrich, Luis and the Editor-in-Chief of *Virus Research*, Brian Mahy, for their continued interest and support to the research on virus evolution over the years.

My thanks go also to the 19 authors who despite their busy schedules have taken time to prepare excellent manuscripts, to Elsevier staff for their prompt responses to my requests, and, last but not least, to Ms. Lucía Horrillo from Centro de Biología Molecular “Severo Ochoa” for her patient dealing with the correspondence with authors and the final organization of the issue.

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Available online 8 December 2004

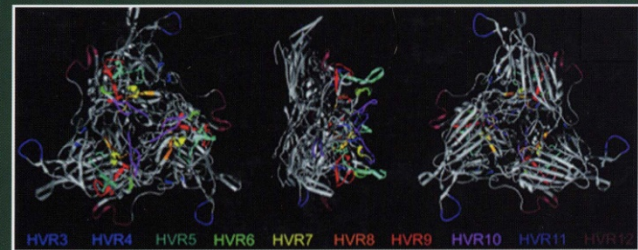


Esteban Domingo

1943 -

SECOND EDITION

ORIGIN AND EVOLUTION OF VIRUSES

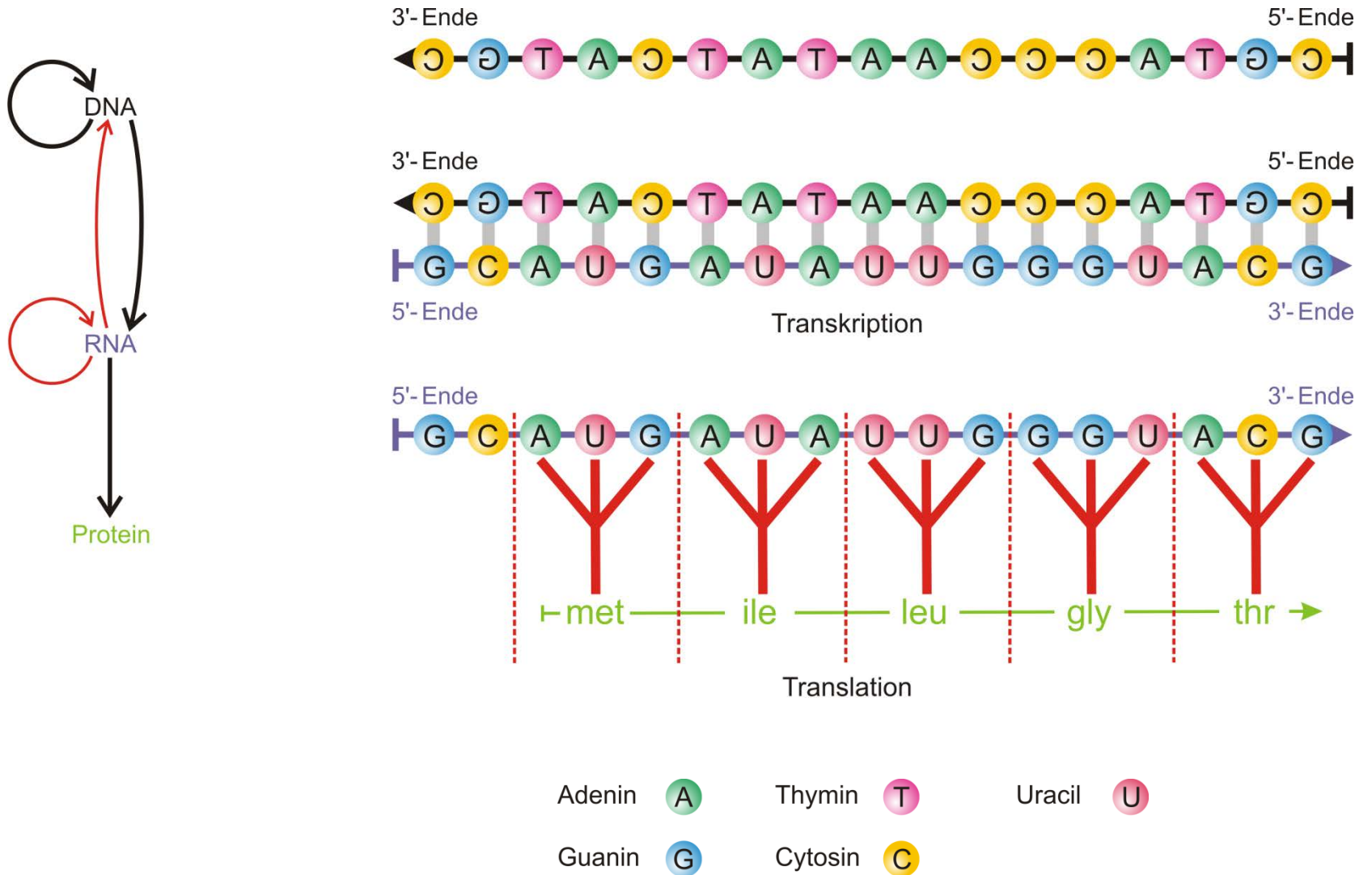


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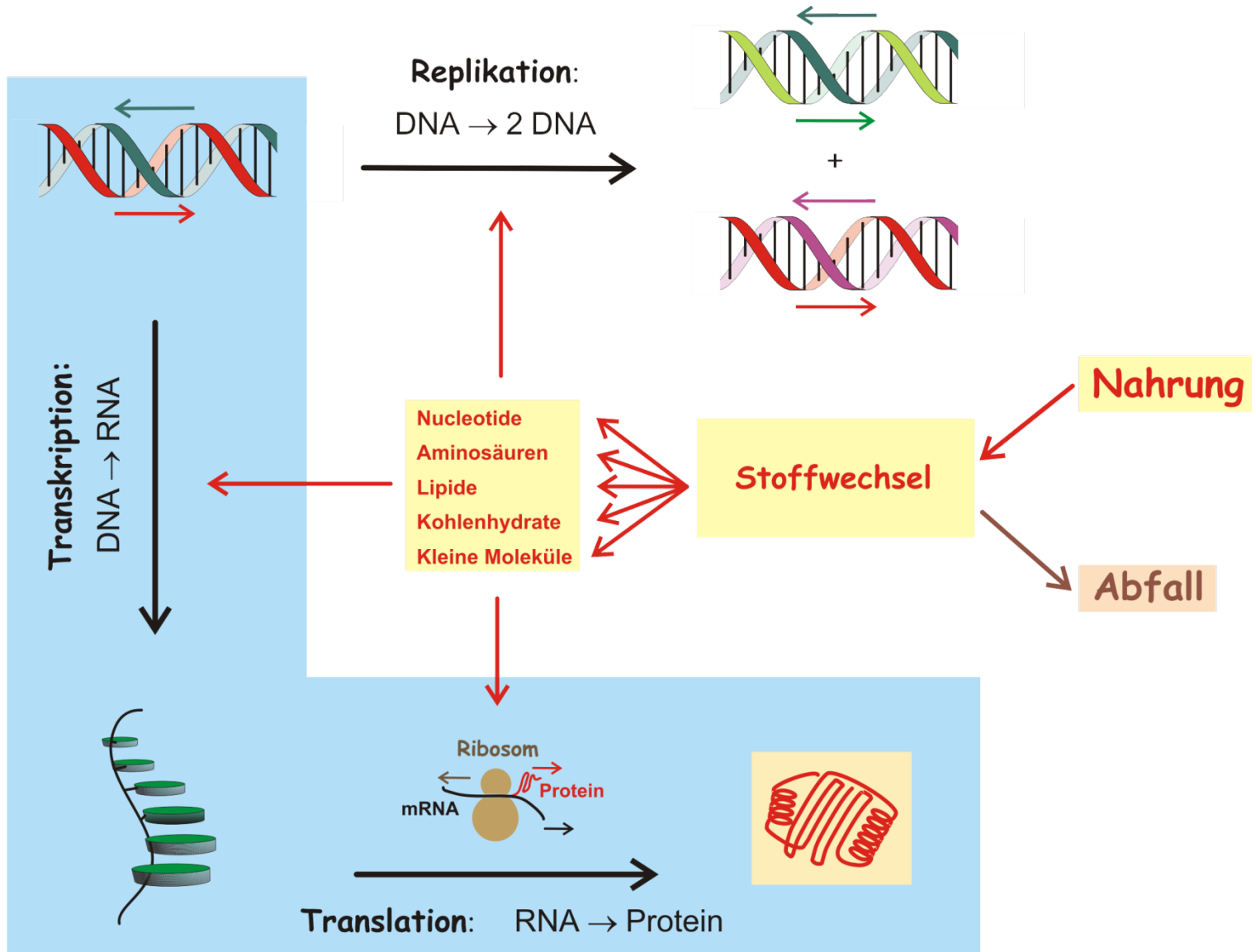


Molecular evolution of viruses

Evolution zu höherer Komplexität



The ,central dogma' of molecular biology



Replizierende Moleküle	⇒	Membranen, organisierte Teilung Moleküle in Kompartments
Unabhängige Replikatoren	⇒	Molekülverkettung, gemeinsame Replikation Chromosomen
RNA als Gen und Enzyme	⇒	Genetischer Code, Ribosom DNA und Protein
Prokaryoten	⇒	Zusammenschluß durch Endosymbiose Eukaryoten
Asexuell vermehrende Klone	⇒	Ursprung der sexuellen Vermehrung Sexuell vermehrende Populationen
Protisten	⇒	Zelldifferenzierung und Entwicklung Pflanzen, Pilze und Tiere
Einzel lebende Individuen	⇒	Entstehung nicht-reproduktiver Kasten Tierkolonien
Primatengesellschaften	⇒	Sprache, Schrift, Kultur, ... menschliche Gesellschaften

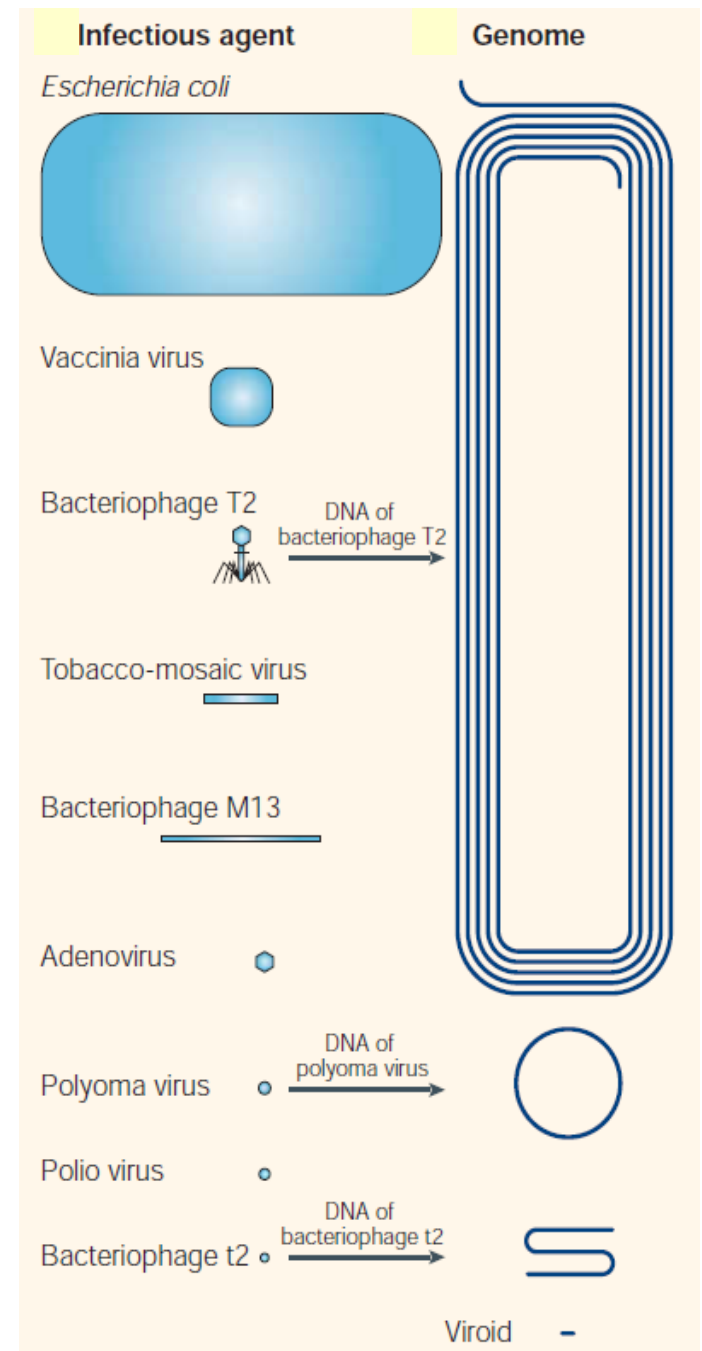
Viroide

Viroids: circular RNAs 246 - 401 nt long
infect inclusively plants

Theodor O. Diener. 2003. Discovering viroids –
A personal perspective.
Nat.Rev.Microbiology 1:75-80.

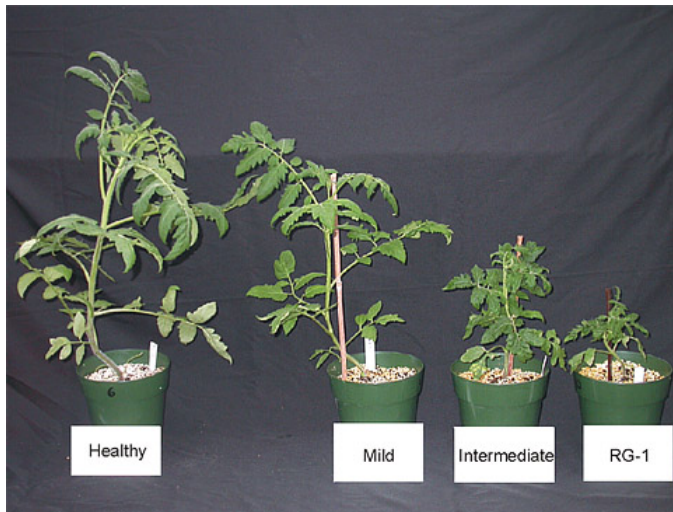
José-Antonio Daròs, Santiago F. Elena, Ricardo Flores.
2006. Viroids: An Ariadne's thread through the
RNA labyrinth. EMBO Reports 7:593-598.

Ricardo Flores *et al.* 2009. Viroid replication: Rolling
circles, enzymes and ribozymes.
Viruses 2009:317-334.





J. Demez. European and mediterranean plant protection organization archive. France



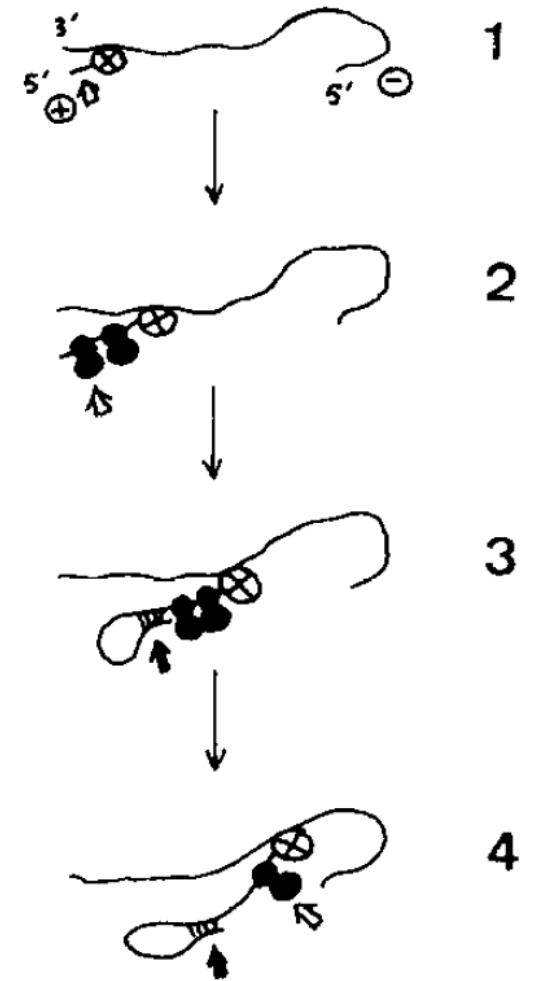
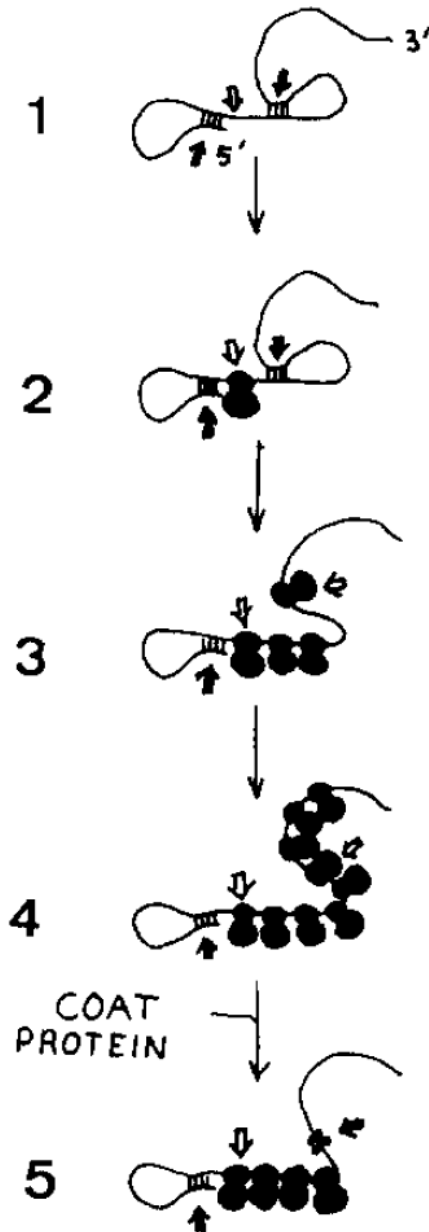
R.W. Hammond, R.A. Owens. Molecular Plant Pathology Laboratory, US Department of Agriculture

Plant damage by viroids

Virusspezies

⊕ REPLICASE
 ● RIBOSOME
 ≡ HYDROGEN-BONDING

↓ INITIATION SITE, OPEN
 ↓ INITIATION SITE, CLOSED



Charles Weissmann. 1974.
 The making of a phage.
FEBS Letters 40:S10-S18.

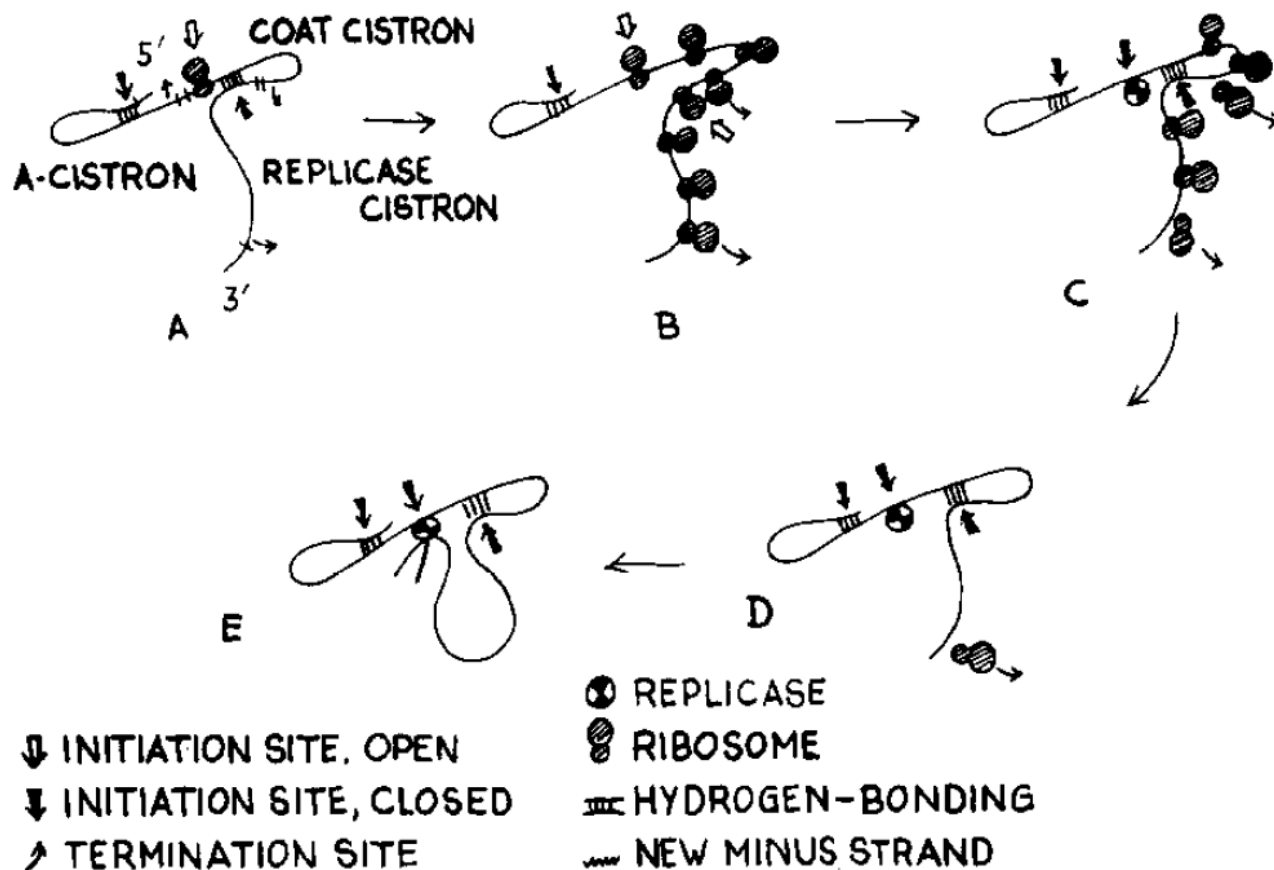
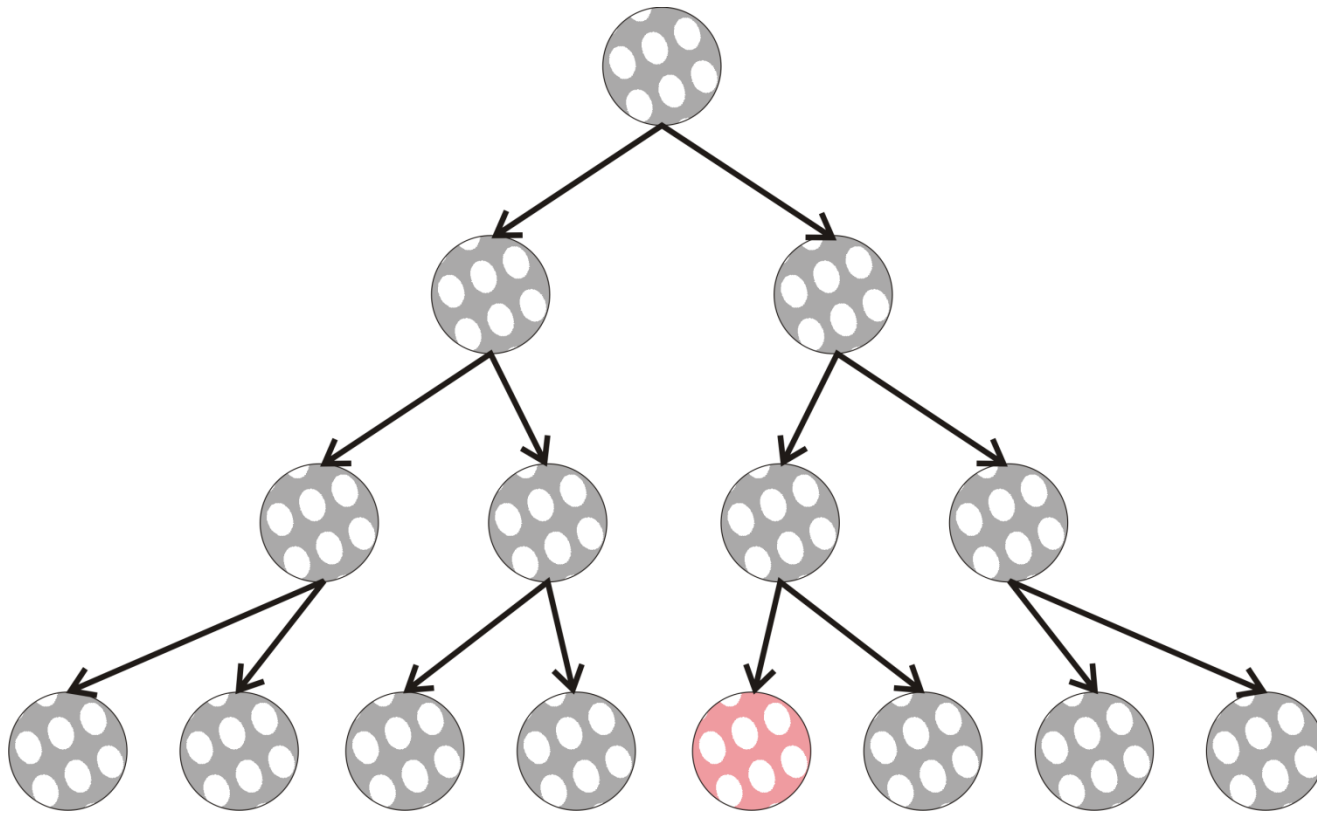


Fig. 3. Transition of phage RNA from polysome to replicating complex – repressor function of Q β viral replicase. (A) Ribosomes attach to the RNA at the coat initiation site. The initiation site of the replicase cistron is unavailable because of the secondary structure of the RNA. (B) Translation of the coat cistron ensues and the initiation site of the replicase cistron is exposed. The replicase cistron is translated.

(C) When replicase becomes available, it attaches to the initiation site of the coat protein and blocks attachment of ribosomes in this position. The RNA refolds, preventing initiation at the replicase cistron. (D) The RNA is cleared of ribosomes. (E) Replicase can now attach to the 3' terminus and initiate synthesis of the minus strand. The A cistron initiation site is at all times inaccessible to ribosomes because of the secondary structure of the mature RNA (cf. fig. 2) (from ref. [64]).

Bakterien

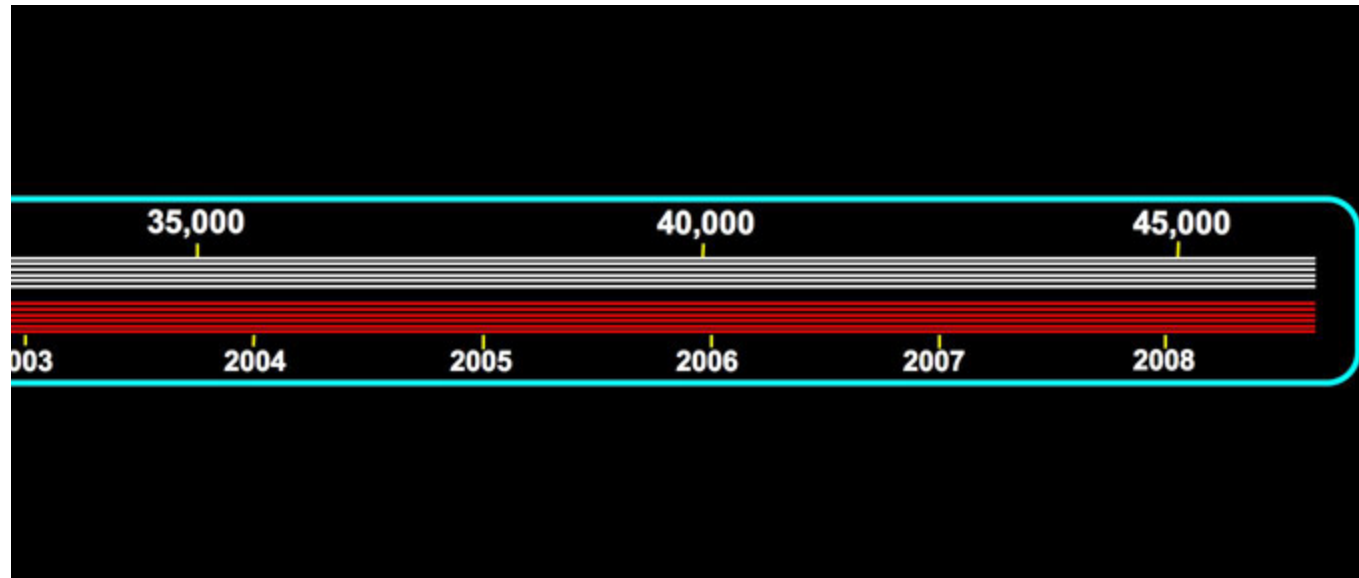
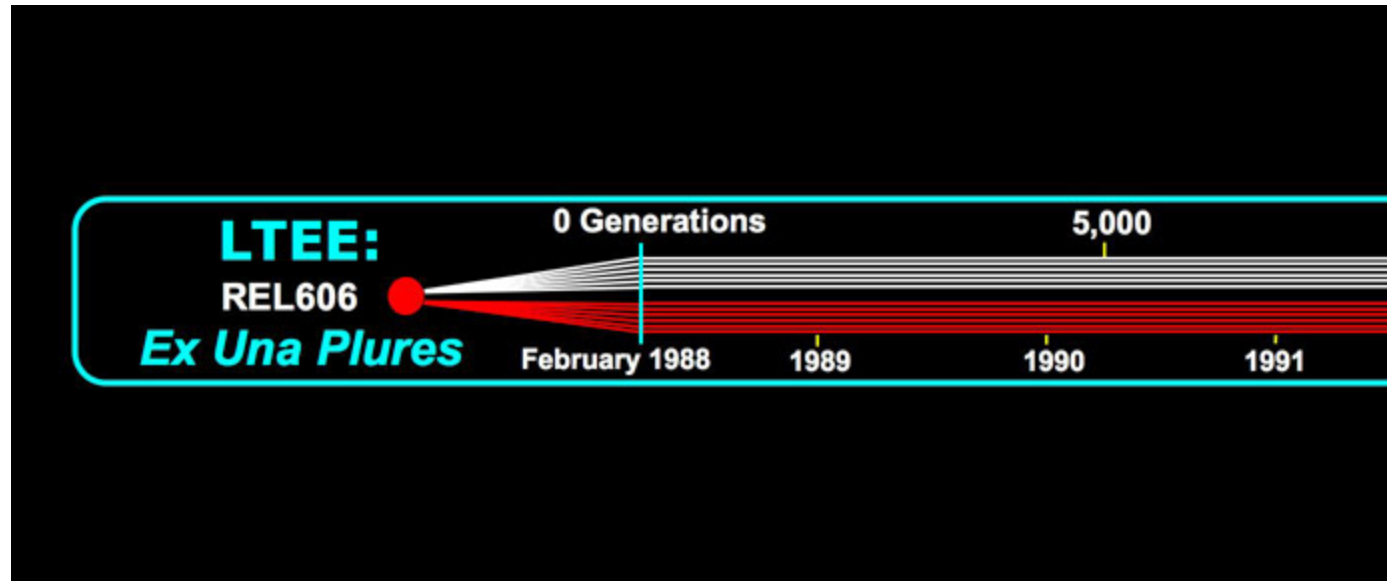


Complex replication dynamics, metabolism, and regulation efficiency are cast into fitness values

Bacterial evolution in cell-lines

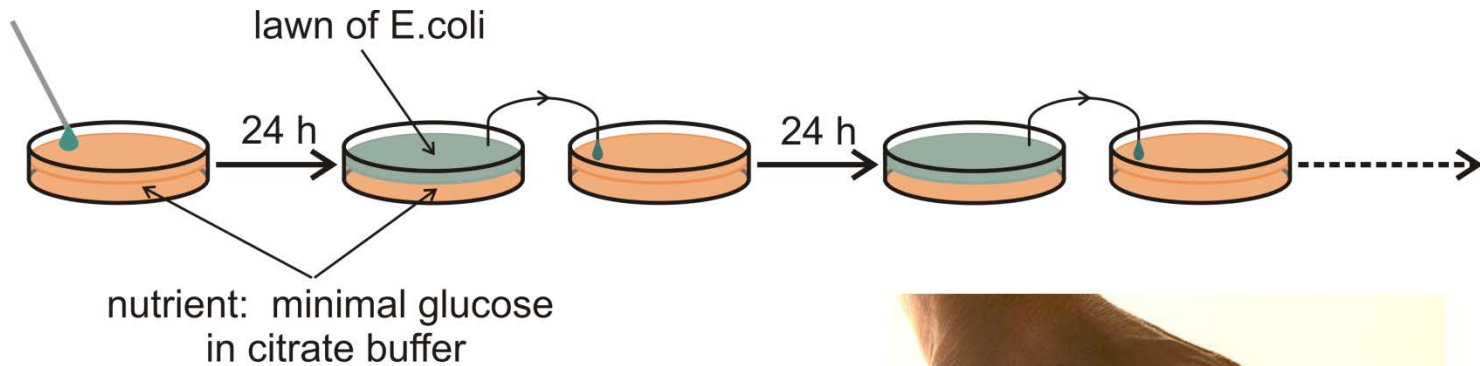


Richard Lenski, 1956 -



Bacterial evolution under controlled conditions: A twenty years experiment.

Richard Lenski, University of Michigan, East Lansing



medium supports $\approx 5 \times 10^8$ bacteria

1 day ≈ 6.67 generations

1 month ≈ 200 generations

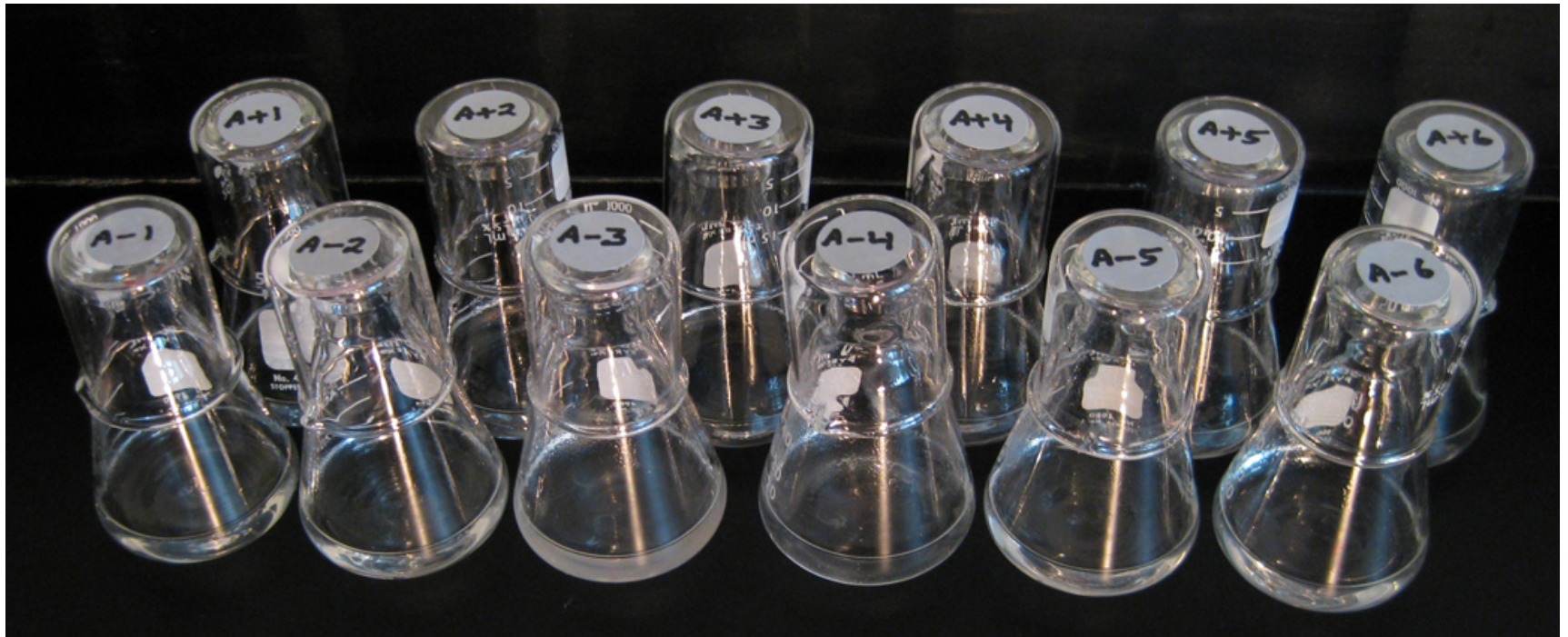
1 year ≈ 2400 generations

Serial transfer of bacterial
cultures in Petri dishes

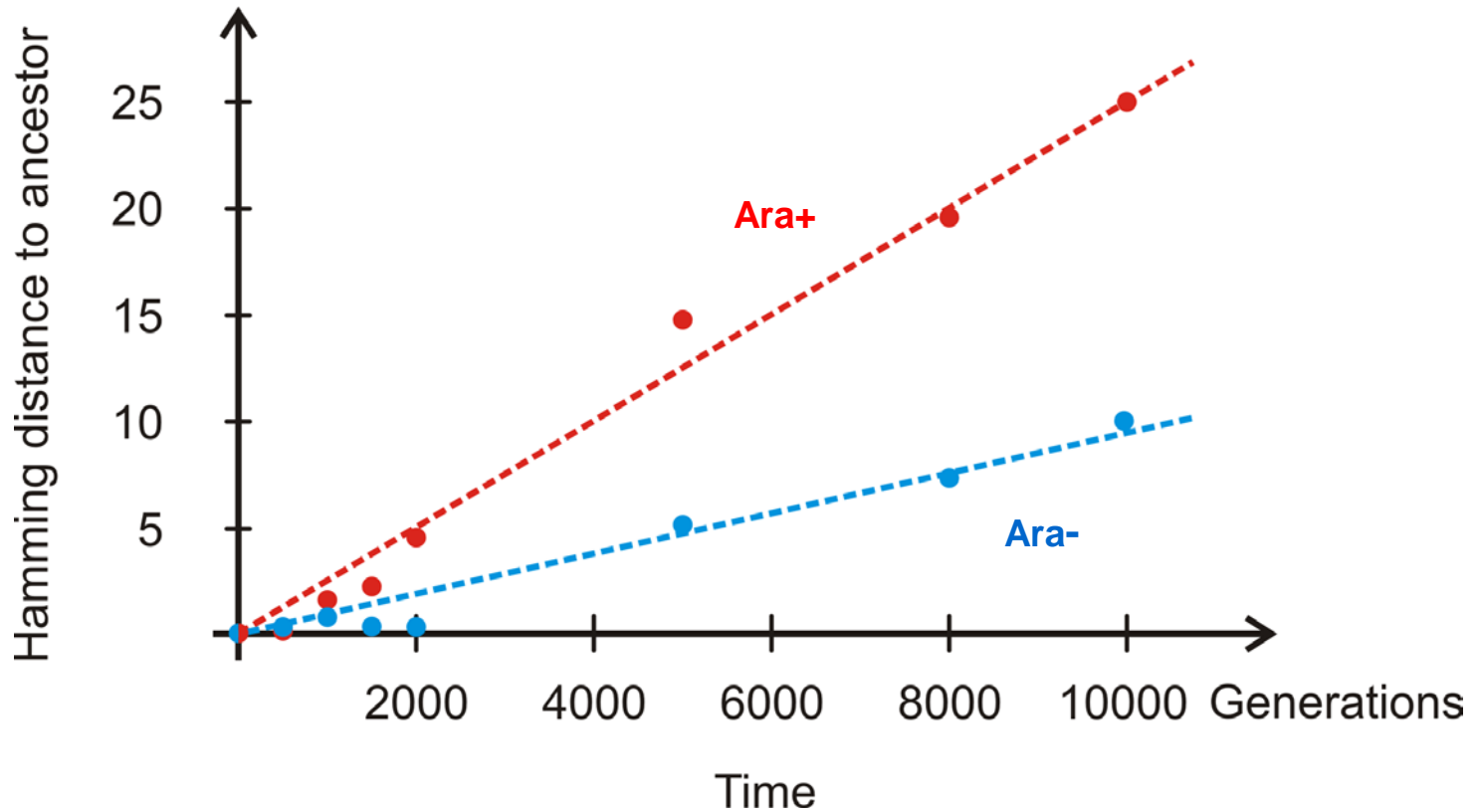


Bacterial evolution under controlled conditions: A twenty years experiment.

Richard Lenski, University of Michigan, East Lansing



The twelve populations of Richard Lenski's long time evolution experiment



Variation of genotypes in a bacterial serial transfer experiment

D. Papadopoulos, D. Schneider, J. Meier-Eiss, W. Arber, R. E. Lenski, M. Blot. *Genomic evolution during a 10,000-generation experiment with bacteria*. Proc.Natl.Acad.Sci.USA **96** (1999), 3807-3812

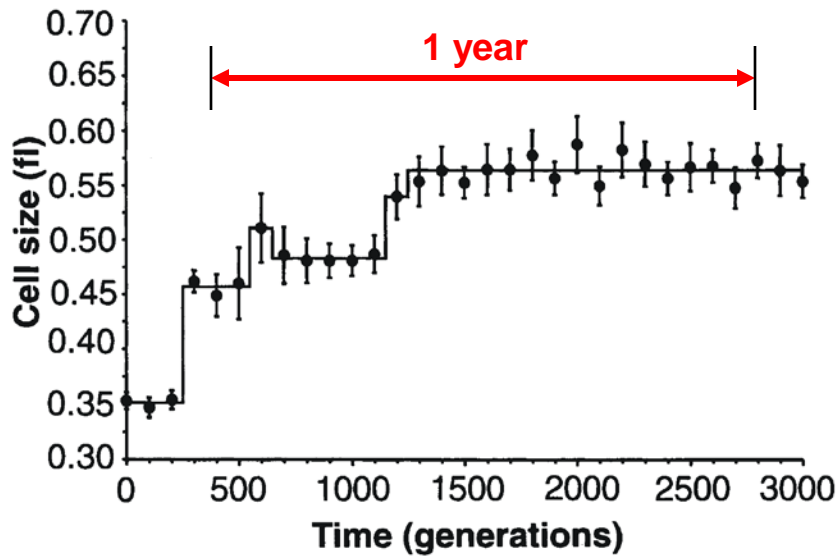


Fig. 1. Change in average cell size (1 fl = 10^{-15} L) in a population of *E. coli* during 3000 generations of experimental evolution. Each point is the mean of 10 replicate assays (22). Error bars indicate 95% confidence intervals. The solid line shows the best fit of a step-function model to these data (Table 1).

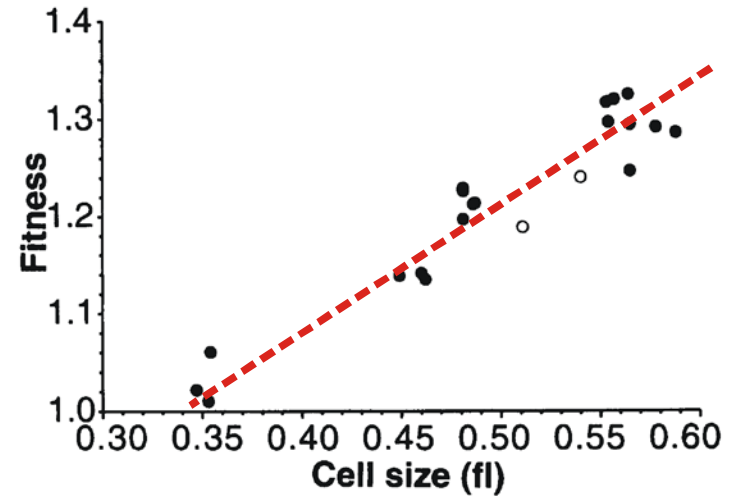


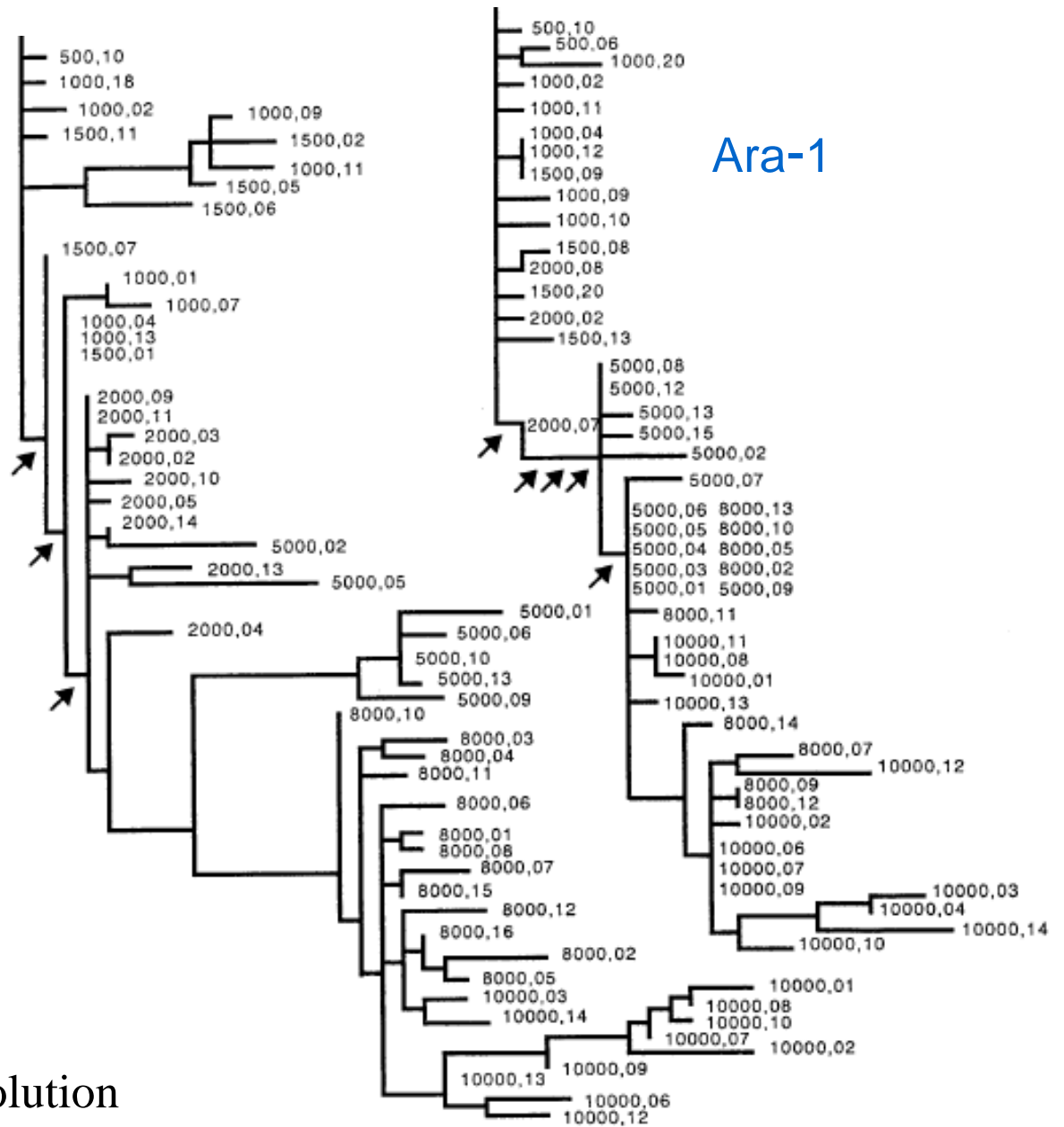
Fig. 2. Correlation between average cell size and mean fitness, each measured at 100-generation intervals for 2000 generations. Fitness is expressed relative to the ancestral genotype and was obtained from competition experiments between derived and ancestral cells (6, 7). The open symbols indicate the only two samples assigned to different steps by the cell size and fitness data.

Epochal evolution of bacteria in serial transfer experiments under constant conditions

S. F. Elena, V. S. Cooper, R. E. Lenski. *Punctuated evolution caused by selection of rare beneficial mutants.* Science **272** (1996), 1802-1804

Ara+1

Ara-1



Phylogeny in *E. coli* evolution



The twelve populations of Richard Lenski's long time evolution experiment
Enhanced turbidity in population A-3

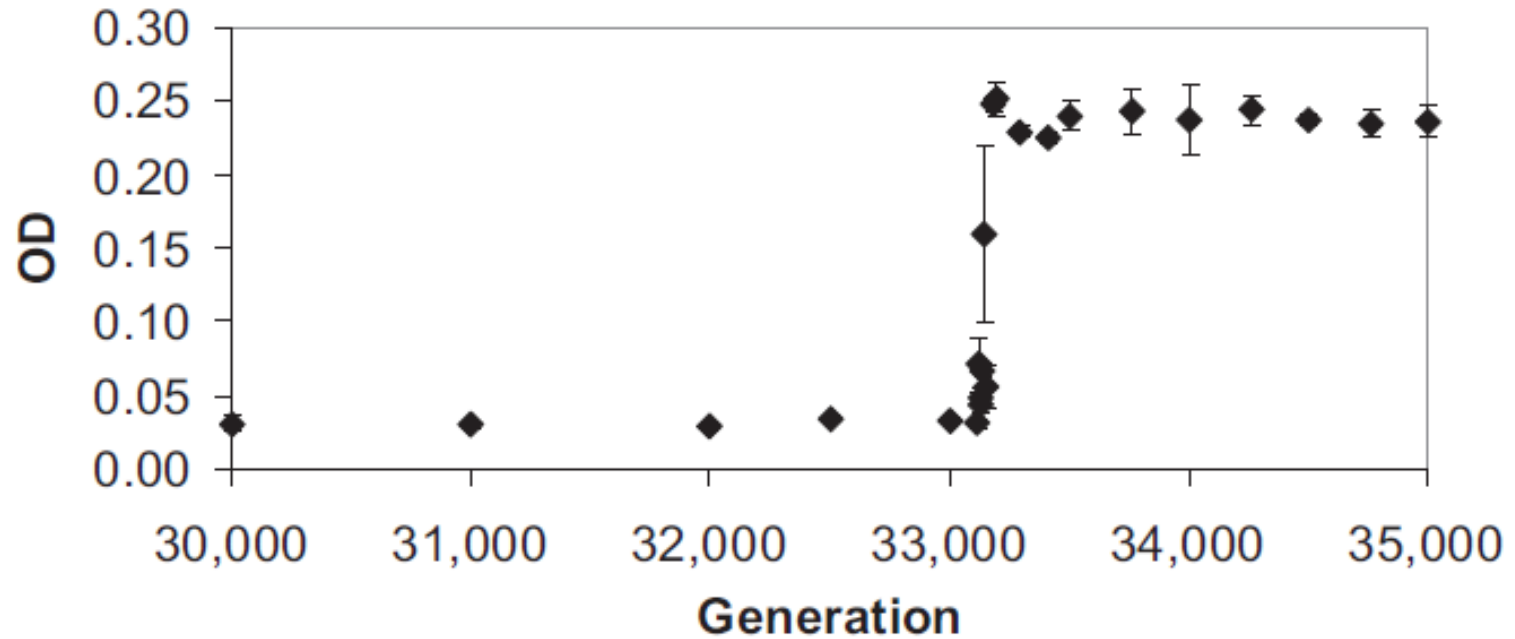


Fig. 1. Population expansion during evolution of the Cit⁺ phenotype. Samples frozen at various times in the history of population Ara-3 were revived, and three DM25 cultures were established for each generation. Optical density (OD) at 420 nm was measured for each culture at 24 h. Error bars show the range of three values measured for each generation.

Innovation by mutation in long time evolution of *Escherichia coli* in constant environment

Z.D. Blount, C.Z. Borland, R.E. Lenski. 2008. *Proc.Natl.Acad.Sci.USA* 105:7899-7906

Table 1. Summary of replay experiments

Generation	First experiment		Second experiment		Third experiment	
	Replicates	Independent Cit ⁺ mutants	Replicates	Independent Cit ⁺ mutants	Replicates	Independent Cit ⁺ mutants
Ancestor	6	0	10	0	200	0
5,000	—	—	—	—	200	0
10,000	6	0	30	0	200	0
15,000	—	—	—	—	200	0
20,000	6	0	30	0	200	2
25,000	6	0	30	0	200	0
27,000	—	—	—	—	200	2
27,500	6	0	30	0	—	—
28,000	—	—	—	—	200	0
29,000	6	0	30	0	200	0
30,000	6	0	30	0	200	0
30,500	6	1	30	0	—	—
31,000	6	0	30	0	200	1
31,500	6	1	30	0	200	1
32,000	6	0	30	4	200	2
32,500	6	2	30	1	200	0
Totals	72	4	340	5	2,800	8

Contingency of *E. coli* evolution experiments

Epilog

Evolution im Licht der gegenwärtigen Molekulargenetik

1. Die Vorstellungen der konventionellen Genetik müssen hinsichtlich der Genregulation entscheidend erweitert werden.
2. Ein Gen wird im Vielzellerorganismus gewebsspezifisch in mehrere verschiedene Proteine übersetzt.
3. Umwelteinflüsse geben Anlass zu Veränderungen des Genoms, welche einige Generationen lang vererbbar sind.
4. Komplexität, Robustheit und Plastizität der Organismen wird erst im Zusammenspiel von Genetik und Epigenetik verstehbar.

Mycoplasma pneumoniae:	Genomelänge	820 000 bp
	# Gene:	733
	# Proteine (ORF):	689
	# tRNAs	37
	# rRNAs	3
	# andere RNAs	4

S. Kühner, V. van Noort, M. J. Betts, A. Leo-Macias, C. Batisse, M. Rode, T. Yamada, T. Maier, S. Bader, P. Beltran-Alvarez, D. Castaño-Diez, W.-H. Chen, D. Devos, M. Güell, T. Norambuena, I. Racke, V. Rybin, A. Schmidt, E. Yus, R. Aebersold, R. Herrmann, B. Böttcher, A. S. Frangakis, R. B. Russell, L. Serrano, P. Bork, and A.-C. Gavin. 2009.

Proteome organization in a genome-reduced bacterium. *Science* **326**:1235–1240.

E. Yus, T. Maier, K. Michalodimitrakis, V. van Noort, T. Yamada, W.-H. Chen, J. A. Wodke, M. Güell, S. Martínez, R. Bourgeois, S. Kühner, E. Raineri, I. Letunic, O. V. Kalinina, M. Rode, R. Herrmann, R. Gutiérrez-Gallego, R. B. Russell, A.-C. Gavin, P. Bork, and L. Serrano. 2009.

Impact of genome reduction on bacterial metabolism and its regulation. *Science* **326**:1263–1268.

M. Güell, V. van Noort, E. Yus, W.-H. Chen, J. Leigh-Bell, K. Michalodimitrakis, T. Yamada, M. Arumugam, T. Doerks, S. Kühner, M. Rode, M. Suyama, S. Schmidt, A.-C. Gavin, P. Bork, and L. Serrano. 2009.

Transcriptome complexity in a genome-reduced bacterium. *Science* **326**:1268–1271.

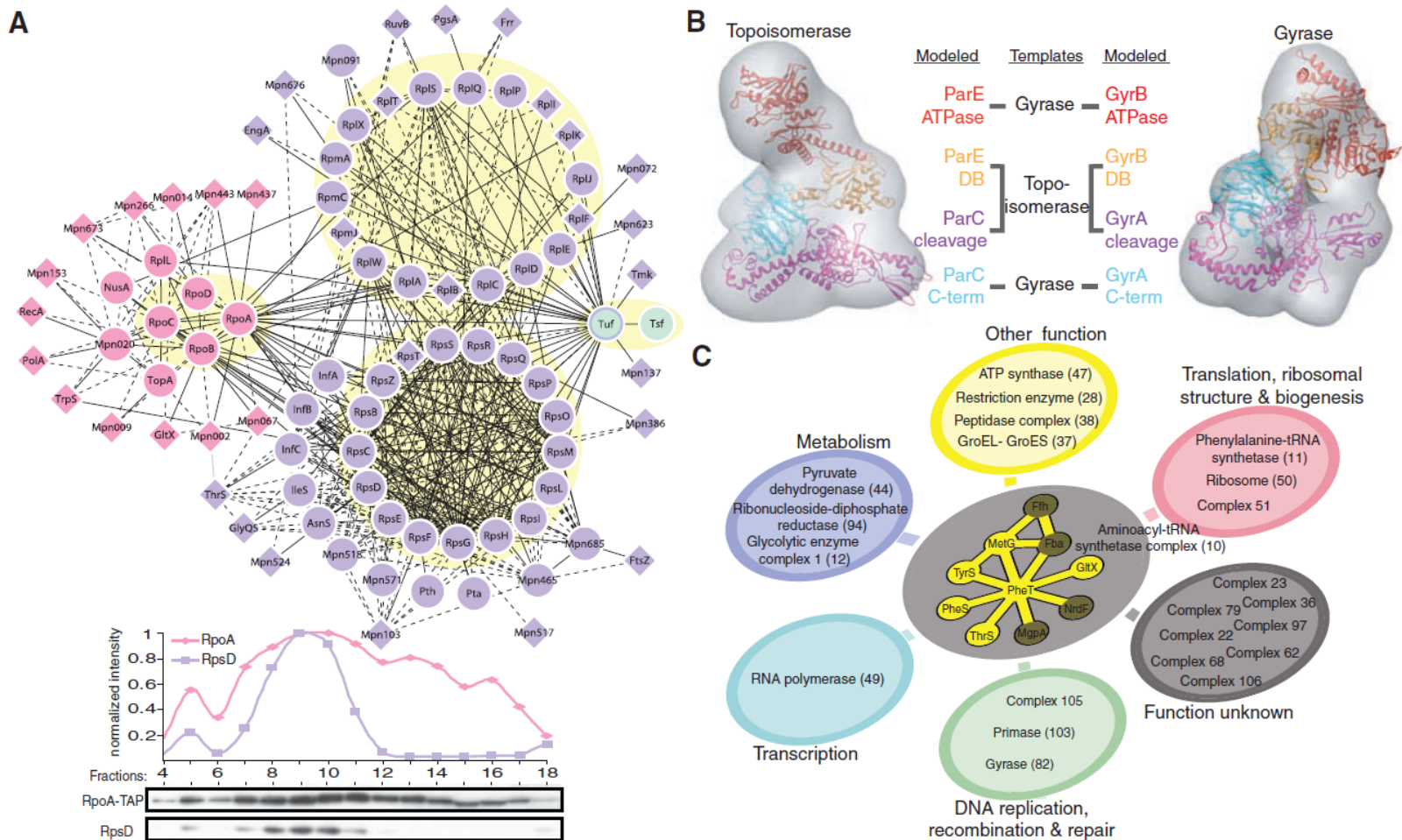


Fig. 3. Higher level of proteome organization. **(A)** The RNA polymerase–ribosome assembly. Core components are represented by circles, attachments by diamonds. The line attribute corresponds to socio-affinity indices: dashed lines, 0.5 to 0.86; plain lines, >0.86. Color code and shaded yellow circles around groups of proteins refer to individual complexes: RNA polymerase (pink), ribosome (purple), and translation elongation factor (green). The bottom graph shows that the ribosomal protein RpsD (23 kD) and the α subunit of the RNA polymerase, RpoA-TAP (57 kD), co-elute in high molecular weight fractions (MD range) during gel filtration chromatography. **(B)** DNA topoisomerase (diameter ~ 12 nm) is a heterodimer in bacteria: ParE (ATPase

and DNA binding domains) and ParC (cleavage and ParC–cleavage domains). The interaction between ParE–DNA–binding and ParC–cleavage domains was modeled by using yeast topoisomerase II as a template [Protein Data Bank (PDB) code 2rgr], and ParE–ATPase and ParC–C-terminal domains were modeled separately on structures of gyrase homologs (PDB 1kij and 1suu). All four domains were fitted into the electron microscopy density. Gyrase (~12 nm) is similarly split in bacteria into GyrA/GyrB, which are paralogs of ParE/ParC, and was modeled and fitted by using PDB 1bjt as a template for the GyrB–DNA–binding and GyrA–cleavage domains interaction. **(C)** Protein multifunctionality in *M. pneumoniae* illustrated with the AARS complexes.

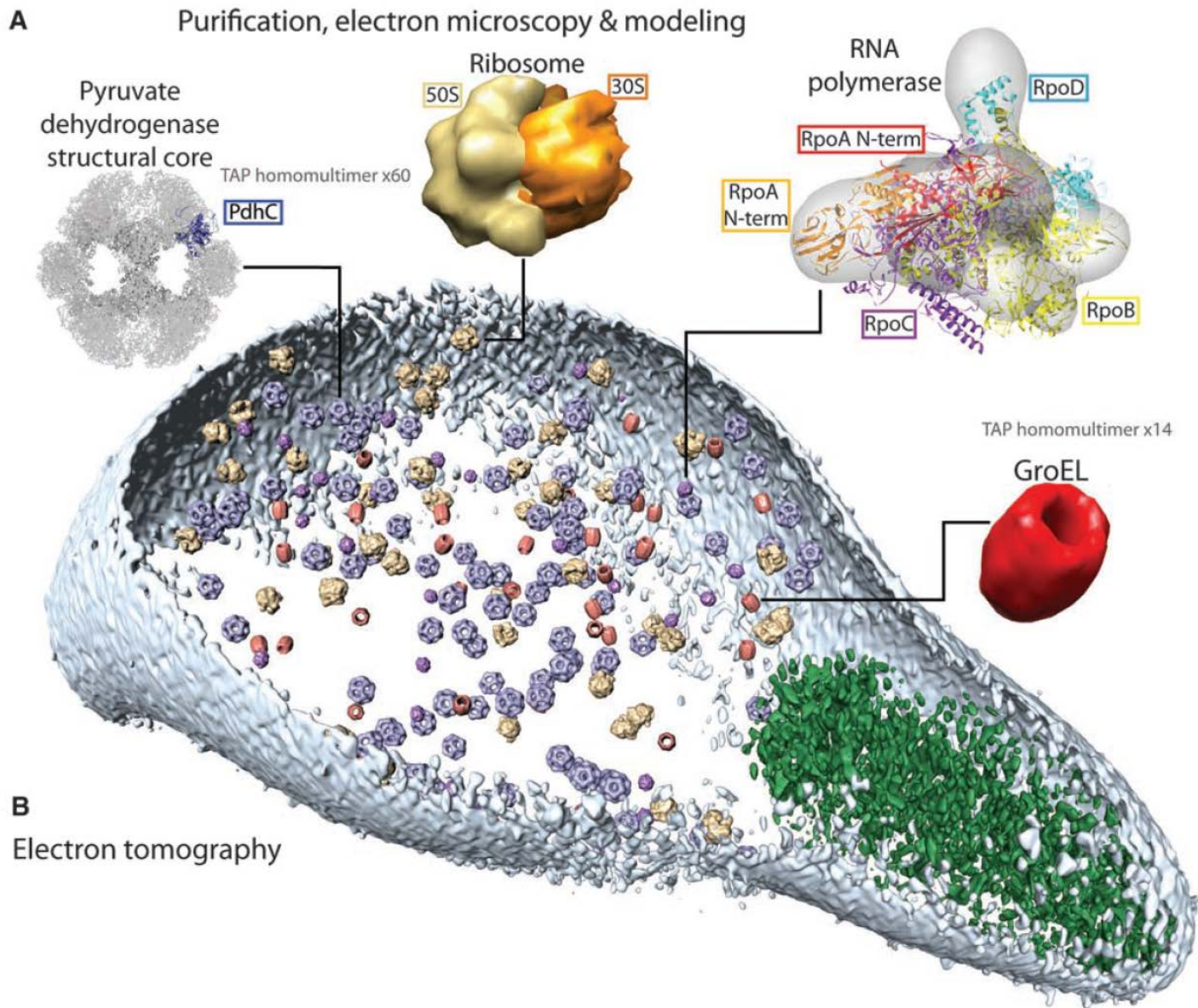
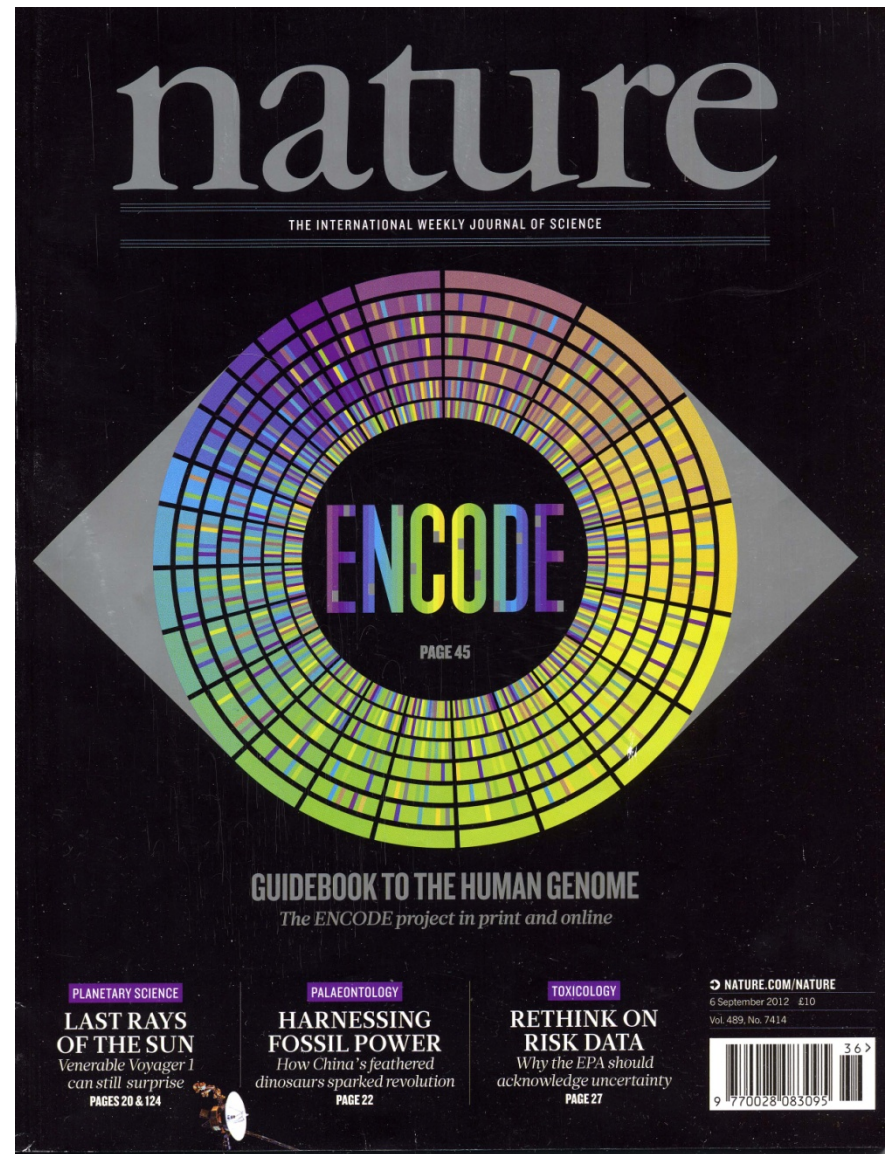


Fig. 4. From proteomics to the cell. By a combination of pattern recognition and classification algorithms, the following TAP-identified complexes from *M. pneumoniae*, matching to existing electron microscopy and x-ray and tomogram structures (**A**), were placed in a whole-cell tomogram (**B**): the structural core of pyruvate dehydrogenase in blue (~23 nm), the ribosome in yellow (~26 nm), RNA polymerase in purple (~17 nm), and GroEL homo-

multimer in red (~20 nm). Cell dimensions are ~300 nm by 700 nm. The cell membrane is shown in light blue. The rod, a prominent structure filling the space of the tip region, is depicted in green. Its major structural elements are HMW2 (Mpn310) in the core and HMW3 (Mpn452) in the periphery, stabilizing the rod (42). The individual complexes (A) are not to scale, but they are shown to scale within the bacterial cell (B).



2007

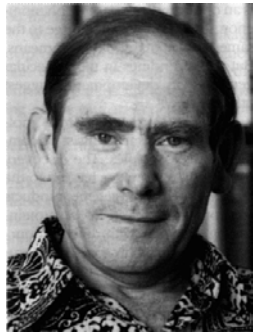


2012

ENCyclopedia **Of** **DNA** **E**lements

Vorteile der molekularen Erforschung des Lebens

1. Komplexe Reproduktionsmechanismen sind erklärbar.
2. Generegulation - basierend auf DNA oder RNA - ist nichts anderes als chemische Kinetik!
3. Epigenetik wird durch die gleichzeitige Betrachtung mehrerer Generationen einfach verstehbar.



What else is epigenetics than a funny form of enzymology ?
Each protein, after all, comes from some piece of DNA.

Sydney Brenner, 1927 -

Danke für die Aufmerksamkeit!

Web-Page für weitere Informationen:

<http://www.tbi.univie.ac.at/~pks>

